

ION AND WATER TRANSPORT IN CELLS AND TISSUES, CHIEFLY OF PLANTS

By

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INTRODUCTION

On changing over from nuclear physics to "biophysics" seven years ago, I spent about a year on odd problems in clinical medicine before coming to biology proper. Another period of six months or so was spent measuring heat production in sea urchin eggs and wondering whether the problems of embryology were suitable for someone with my training. Then I came across the work of A.L. Hodgkin on ion permeability and nerve action mechanisms and decided to work somewhere in the "permeability field". This thesis is a record of most of the work in which I have taken a leading part since then. Not included are some unpublished, and not very good, work on ion transport in Xenopus embryos, on ion uptake by potato discs and on ion transport in Ulva lactuca, and another unpublished manuscript (with Dr. E.A.C. MacRobbie) on the kinetics of ion exchange between animal tissues of various shapes - flat muscle, cylindrical nerve bundle, etc. I have also not had time to include one or two other papers in active preparation.

Under the influence of Hodgkin's papers, the first ion transport work done was with animal tissues; the paper with Krnjević on "The Rate of Exchange of ^{24}Na in Cat Nerves" describes one of the few published attempts made to measure ion transport in vertebrate nerve and muscle; it is also the only work I know of in which a correct kinetic analysis is made of the ion exchange between external solution and a tissue which contains an extracellular space, (this analysis was developed with Dr. MacRobbie).

Soon an interest in ion distribution and transport in plants developed. At first, still guided by animal physiology, I worked on marine algae - Rhodymenia palmata and Ulva lactuca - , because they live in a high salt concentration; therefore ion flux measurements can be made using tracers under what are known to be physiological conditions. It was also felt that some of the features of ion transport in animal tissues would be found in marine algae and indeed they were.

The greater part of this work is described in the paper: "Sodium and Potassium Distribution and Transport in the seaweed Rhodymenia palmata (L) Grev." (The co-author of this and the next paper, Dr. E.A.C. MacRobbie, was a research student working under my supervision.)

Although this work on Rhodymenia proved to be interesting and valuable, (e.g. Rhodymenia now holds the record for the ratio $\frac{[Na]_o}{[K]_o} / \frac{[Na]_i}{[K]_i}$ - about 1500 -, and the spectacular effect of light on the K flux was discovered), and despite the fact that Rhodymenia should be ideal material for studying the metabolic connections of ion transport, attention was transferred away from complex, inhomogeneous, tissues to single cells. It is a consequence (and not necessarily an advantage) of a long training in the physical sciences, that I constantly want to choose "simple" systems to work with so that the measurements made mean something quite definite in a physical sense - indeed, if possible, are meaningful at the molecular level.

The single cell chosen was Nitellopsis obtusa and it was selected because it is an ecorticate member of the Characeae living in brackish water. Again I was influenced, in this choice, by the possibility of making ion flux determinations under physiological conditions. The results of this work - possibly the most important I have so far published - is described in the paper "Ion Transport in Nitellopsis obtusa". The chief result of this work was the unequivocal proof of the existence of sodium and chloride "pumps" in this organism. Such a proof is only available for complex animal membranes, such as frog skin, which separate two purely aqueous (i.e. non-colloidal) solutions.

Until 1958, all my work had been done in more or less complete isolation; last year, however, thanks to a Nuffield - Royal Society Commonwealth bursary, I was able to spend four months working with Dr. A.B. Hope in the C.S.I.R.O. Plant Physiology Unit attached to the Botany Department, University of Sydney. The two papers, "The Water Permeability of Cells of Chara australis" and "Ionic Relations

of Cells of Chara australis. I. Ion Exchange in the Cell Wall", represent part of the work I did there; there is also a second paper on "Ionic Relations of Cells of Chara australis" in active preparation, but not yet in a suitable state to include in this thesis. Dr. Hope and I shared the experimental work, but I think it would be fair to say that most of the ideas involved are mine. Since sending these two papers to the press, I have had further ideas arising from these papers and have included these in the thesis under the titles: "Notes on Water Permeability" and "Notes on Free Space (chiefly Donnan Free Space) in Plant Tissues". The latter can also be looked upon as a (very) extended version of a paper I have been invited to give at the IX International Botanical Congress in Montreal in August, 1959.

I have also presented three papers describing joint work done with Mr. Simpson and Dr. Verma, of the East of Scotland College of Agriculture, on phosphate uptake by growing crops. This co-operation with Mr. Simpson is continuing and we are at present making a biochemical study of the fate of the phosphate in the plant, so as to try and find the physiological basis of the reduction in yield at high phosphate levels.

This record of the work I have carried out on uptake and permeability problems has, of necessity, been joint work; for during this period I have had the task of building up a Biophysics Department (with a load of elementary teaching) from scratch. However, in general, I have been the dominant partner in the published work and done my fair share of the experimental work. The two "Notes" I have included represent an attempt to substantiate this claim.

I also present, as additional support, four papers from my earlier period as a nuclear physicist, much of which time was spent in charge of, and rebuilding, the Cambridge cyclotron and in building up the Edinburgh High Voltage laboratory.

8th April 1959

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THE RATE OF EXCHANGE OF ^{24}Na IN CAT NERVES

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The present investigation was begun in an attempt to verify a result of Manery & Bale (1941) that Na in mammalian (rabbit) nerve exchanges only very slowly with plasma Na *in vivo*. As the investigation proceeded it became clear that roughly quantitative information concerning the flux of Na across the nerve surface might be obtained. However it did not prove possible to make sufficiently accurate quantitative studies of the Na flux *in situ*, so a number of experiments on the rate of loss of ^{24}Na from desheathed cat nerves *in vitro* were carried out. From the results of the latter experiments estimations of the Na efflux were obtained.

METHODS

All nine cats used in the experiments were anaesthetized with intraperitoneal pentobarbitone sodium (Nembutal), small doses being subsequently given intravenously to maintain a light level of anaesthesia.

The solution injected was a mammalian saline (0.9% NaCl), made up from irradiated NaCl and obtained from the Atomic Energy Research Establishment, Harwell. At the time of injection, its activity varied between 0.40 and 0.50 mc/ml. The amount of solution injected was 5-7 ml., equivalent to a total of 2.15-3.50 mc of ^{24}Na .

Experimental procedure

^{24}Na exchange *in situ*. The solution was injected into a femoral or a jugular vein and washed in by a few ml. of inactive saline; the time required for this operation was about 1 min. At intervals after the injection, 4-5 cm long portions of the posterior tibial, the lateral popliteal, or, sometimes, the medial popliteal nerves were removed from one or the other hind limb; a sample of carotid arterial blood was obtained simultaneously. The nerves were rinsed very quickly in inactive saline to remove superficial contamination with blood, and, after lightly blotting with filter-paper, were immediately weighed in platinum crucibles of known weights. The crucibles were placed in an air-oven at about 100° C for at least 5-6 hr, and, after cooling in a desiccator, weighed again. They were then left overnight in a muffle furnace at a temperature not exceeding 550° C. In the morning, the ash was dissolved in 5 ml. nitric acid (0.75 N). A portion of this solution (about 3 ml.) was diluted about $\times 50$, and the Na and K content then estimated by means of an Evans Electro-selenium flame photometer, with an accuracy rather better than 5% for Na and 10% for K. Details of the method and its accuracy are given in Krnjević (1955). The rest of the nitric acid solution (about 2 ml.) was used to determine the ^{24}Na content of the nerve; this was always done within 24 hr of the end of the experiment. The heparinized blood samples were centrifuged soon

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after removal, and 1.00 ml. samples of plasma pipetted into platinum crucibles; they were then submitted to a procedure exactly similar to that described above.

There were four nerve and blood samples in each experiment, and they were taken at intervals after the injection which were not the same throughout the series. The first was usually after about 5 min; the 2nd and 3rd were after intervals varying between 10 and 60 min, and the 4th was either after 60 or 75 min, or after some 5–5½ hr.

Loss of ^{24}Na from desheathed nerves in vitro. At least 4–5 hr after the injection of the active saline, one of the lateral popliteal nerves was dissected out and rapidly desheathed. About 6 cm of desheathed nerve was then fixed in a stainless steel clamp, which grasped firmly the two cut ends and the nerve and clamp quickly weighed. Both were then plunged into a bath containing 10.0 ml. inactive Tyrode solution, stirred and oxygenated at 37° C. At intervals of about 5 min initially later of 30 min, this bathing solution was replaced by 10.0 ml. fresh, inactive, Tyrode solution. The duration of this part of the experiment was 3 hr, and in this time twelve 10.0 ml. samples were collected. The radioactivity of these samples was always determined on the same day. A more complete description of the desheathing procedure, and the diffusion apparatus and method, is given by Krnjević (1955).

The composition of the Tyrode solution used was derived from one described by Strangeway (1924):

Na	149.2 m-mole	HCO_3	11.9 m-mole
K	2.7 m-mole	H_2PO_4	0.4 m-mole
Ca	1.8 m-mole	Glucose	100 mg
Mg	0.5 m-mole	Water to	1.0 l.
Cl	144.2 m-mole		

2% CO_2 in the oxygen bubbled through the solution, maintained the pH at about 7.4.

At the end of the experiment, the nerve was weighed again after blotting lightly, and the portions were kept for (1) Na and K determinations, and counting of activity; (2) confirmation of the effectiveness of the desheathing by a histological examination of paraffin sections, after fixing in Flemming's solution.

Determination of the volume/surface area ratio. This was carried out by measuring the internal diameters of all fibres in photomicrographs of paraffin sections of lateral popliteal nerves. The nerves had been fixed in Flemming's solution and dehydrated in dioxan. The magnification at which the photographs were taken was about $\times 700$. According to Sanders (1947) the correction for the shrinkage of fibres caused by dehydration and paraffin embedding, after fixation in Flemming's solution, is not greater than 7%. This correction has been ignored in the present calculations.

Counting procedures. Using a 0.100 ml. graduated pipette, about 0.070 ml. of the ashed nerve solution was pipetted on to a standard counting planchette. The drop of solution was evaporated to dryness and a measure of the amount of radioactivity obtained by counting, in a standard geometrical arrangement, with a GM 4 end-window counter and standard equipment (A.E.R.E. Type 1033 A power unit, E. K. Cole Type N 526 scaler and A.E.R.E. Type 1014 A probe unit). The measure of radioactivity, in counts/min, was corrected for lost counts (due to the finite 'dead time' of the GM 4 and probe unit), background counts and decay of the ^{24}Na . No correction was necessary for source thickness.

The radioactivity of the plasma samples was determined in a similar way.

The radioactivity of the 10 ml. liquid samples collected during the *in vitro* efflux experiment was determined by counting in a M 6 liquid counter. This method gave a perfectly standard geometrical arrangement and the factor to convert counts/min. ml. in this geometry to counts/min. ml. in the GM 4 geometry was determined experimentally. The same counting equipment was used with both the M 6 and the GM 4 counters, and the usual corrections for dead time, background and decay were always made.

In all cases, at least 1000 counts were recorded so that the standard deviation was never more than 3%.

RESULTS

Rate of change of specific activity of nerve Na in situ

The specific activity (S) of a nerve sample was calculated from its total Na content and its activity, and the Na content and activity of the corresponding plasma sample. The specific activity of the latter was taken as 100%.

TABLE 1. The mean values of the specific activity of cat nerves at intervals after the intravenous injection of ^{24}Na saline. The corresponding activity of plasma is 100%.

Time (min)	Mean activity (%)	S.E. of mean	No. of nerves
5	45.2	9.0	6
10	55.2	9.2	4
21	71.0	6.0	3
33	70.8	7.0	5
68	88.5	10.4	8
330	101.1	15.0	4

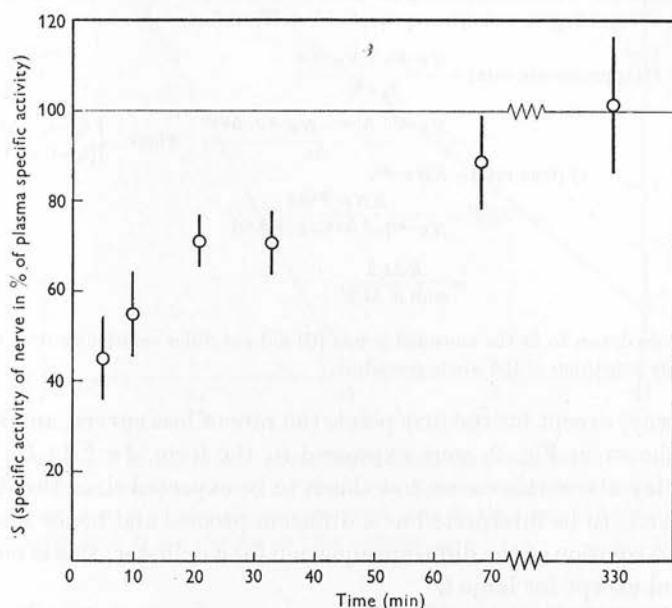


Fig. 1. Values of S , the mean specific activity, of cat nerves removed at various intervals after the intravenous injection of ^{24}Na saline, plotted as a function of time after removal. The specific activity of plasma is taken as 100%. The vertical lines show \pm S.E. of the mean.

All the data obtained in this way were grouped according to the time of removal after injection; it was possible to arrange thirty results from nine cats in six groups, each containing at least three results. The averages of these groups, together with the S.E.'s, are given in Table 1. The values of S , plotted as a function of time, are shown in Fig. 1.

Rate of loss of ^{24}Na from desheathed nerves in vitro

The method employed, which was similar to that of Levi & Ussing (1948) gave values for the loss of ^{24}Na during various time intervals. It was necessary to convert these figures into rates of loss of ^{24}Na at various times.

The radioactivity in counts/min of each 10 ml. sample was a measure of the amount of ^{24}Na which had left the nerve in a given interval of time, say from t_1 to t_2 sec. The number of counts/min divided by $t_2 - t_1$ gives an approximate measure (P) of the rate of loss of ^{24}Na at time $(t_1 + t_2)/2$ sec. To find more accurate values of the rates, the following procedure was adopted. P was plotted on semi-logarithmic graph paper as a function of time $(t_1 + t_2)/2$. Except for the first point, these approximate rates fitted two straight lines, so that the function was of the form $Ae^{-K_1 t} + Be^{-K_2 t}$. Using the values of K so determined, a more accurate value (Q) of the rate was found by multiplying P by the factor

$$\frac{K(t_2 - t_1)/2}{\sinh K(t_2 - t_1)/2}$$

derived as follows:

the observed number of counts appearing in the interval t_1 to $t_2 = Ne^{-Kt_1} - Ne^{-Kt_2}$ assuming that the ^{24}Na is lost according to a simple exponential law. Therefore

$$P \text{ (approximate rate)} = \frac{Ne^{-Kt_1} - Ne^{-Kt_2}}{t_2 - t_1}$$

$$= \frac{Ne^{-K(t - \Delta t/2)} - Ne^{-K(t + \Delta t/2)}}{\Delta t}, \quad \text{where } \begin{cases} t_2 - t_1 = \Delta t \\ (t_1 + t_2)/2 = t \end{cases}$$

$$Q \text{ (true rate)} = KN e^{-Kt}.$$

Therefore

$$Q/P = \frac{KN e^{-Kt} \Delta t}{Ne^{-Kt} [e^{K\Delta t/2} - e^{-K\Delta t/2}]}$$

$$= \frac{K\Delta t/2}{\sinh K\Delta t/2}.$$

Straight lines drawn to fit the amended points (Q) did not differ sufficiently from the original lines to justify repetition of the whole procedure.

In this way, except for the first point, the rate of loss curves, an example of which is shown in Fig. 2, were expressed in the form $Ae^{-K_1 t} + Be^{-K_2 t}$. The first point lay above this curve, but this is to be expected since the 'fast' part of the curve is to be interpreted as a diffusion process and hence must be expressed as a solution of the diffusion equation for a cylinder; this is not a simple exponential except for large t .

The values of K deduced from the slopes of curves based upon five experiments are shown in Table 2. The mean value of the slow K (K_s) was $(4.35 \pm 0.19) \times 10^{-4} \text{ sec}^{-1}$, and of the fast K (K_f) $(32.2 \pm 2.1) \times 10^{-4} \text{ sec}^{-1}$. (The quoted errors are standard errors of the means.)

Fraction of ^{24}Na in the slow component. The equations of the five curves were integrated and the fraction of ^{24}Na in the slow component calculated from the constants of the new equations, suitable corrections being made to allow for the first point of the fast curve. The mean value was 23.9% of the total ^{24}Na with a S.E. of 4.9.

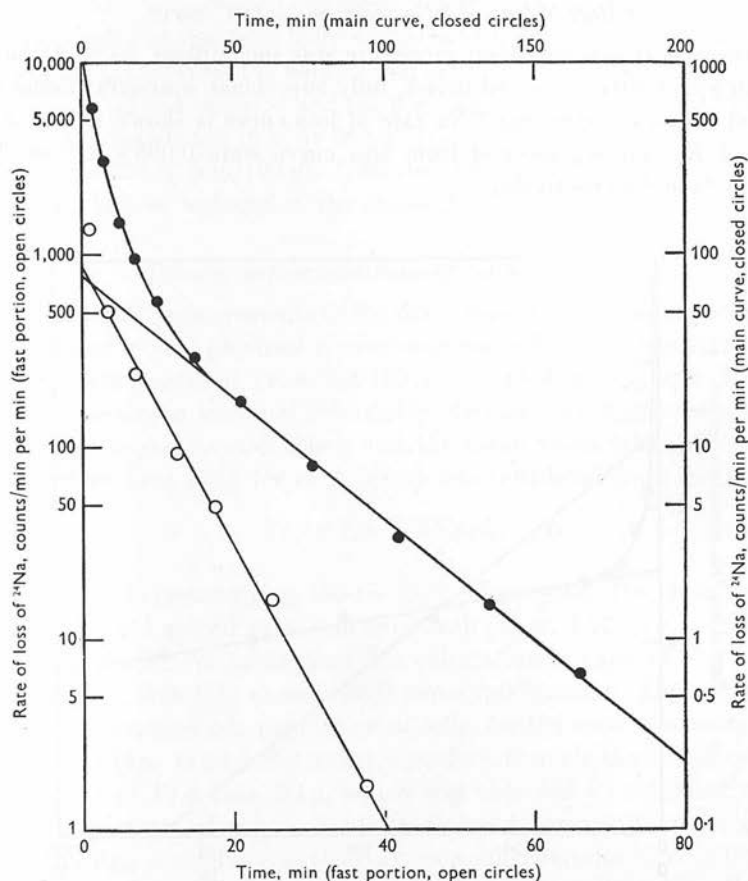


Fig. 2. ●—●, Curve of rate of loss of ^{24}Na from a desheathed cat nerve at various times after being placed in a Tyrode solution. ○—○, Curve of fast component of the main curve, obtained by subtraction of straight line produced back to time zero. The two curves have different scales both in the abscissa and the ordinate.

TABLE 2. Values of K_s and K_f , the rate constants of the slow and fast components governing the loss of ^{24}Na from desheathed cat nerves *in vitro*. K_f was deduced from the straight portion of each diffusion curve.

$10^4 K_s$ (sec^{-1})	$10^4 K_f$ (sec^{-1})
4.25	32.6
3.93	28.7
4.92	28.1
4.75	31.5
3.91	39.9

Rate of loss of ^{24}Na from an 'intact' nerve

In one case the desheathing procedure was incomplete, so that the perineurium apparently remained intact, only superficial, epineural tissue being removed. The corresponding ^{24}Na rate of loss curve is shown in Fig. 3. The values of K_s and K_F derived from this curve were $0.995 \times 10^{-4} \text{ sec}^{-1}$ and $48.2 \times 10^{-4} \text{ sec}^{-1}$ respectively.

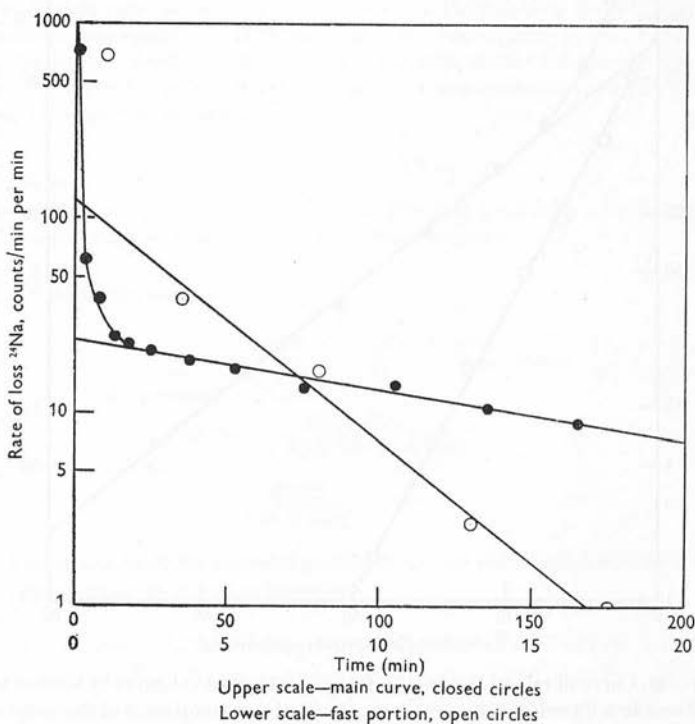


Fig. 3. —●—●, Curve of rate of loss of ^{24}Na from an 'intact' cat nerve at various times after being placed in a Tyrode solution. —○—○, Curve of fast component of the main curve, obtained by subtraction of straight line produced back to the time zero. The two curves have different time scales.

Change in weight of desheathed nerves in Tyrode

Of the five desheathed nerves studied, four were weighed before and after the *in vitro* experiment. All showed an increase in weight which varied between 15 and 49% of the initial weight (mean value: 35%). The average water content of the nerves after the experiment was 74.8% and the Na and K concentrations were 112.6 and 32.4 m-mole/kg nerve respectively.

^{24}Na space in cats

The proportion of the total body weight which may be considered as ^{24}Na space was calculated from the volume and activity of the ^{24}Na saline injected, the activity of plasma samples, and the body weight. It was assumed that the Na was singly distributed in body water. The mean value of the ^{24}Na space in eight cats was 36.5% (ml./100 g). (The standard error of this mean value was 3.6.) In two kittens included in the above, the values were 43.6 and 58.1%.

Volume/surface area ratio of cat nerve fibres

Three groups of measurements of the diameters of axons in different photomicrographs of lateral popliteal nerves were made by two observers. Every visible fibre was measured, i.e. about 150 in each photomicrograph. When the sections of the axons were not reasonably circular, two measurements were made at right angles to each other, and the mean value taken. The overall volume/surface area ratio for each group was calculated from the following equation:

$$V/A = \sum_i n_i d_i^3 / 4 \sum_i n_i d_i,$$

where d_i is the diameter and n_i the respective frequency. The three values of the ratio obtained agreed very well with each other: 1.97×10^{-4} , 2.15×10^{-4} and 2.20×10^{-4} cm. For subsequent flux calculations a value of V/A equal to 2.1μ was taken, but this choice needs some qualification. The nerve fibres contain an inhomogeneous population of cells, for the axon diameters varied between 1 and 16μ . It might be better, therefore, to use in the flux calculations a larger value of V/A than 2.1μ , which was obtained by summing over all axons. For example, if only axons with diameters greater than 5μ are considered then V/A is about 2.8μ ; these axons would comprise about 80% of the total volume and hence, presumably, 80% of the intracellular Na. However, throughout this paper the volume/surface area ratio will be taken as 2.1μ , but the correct value to use could be as high as 3μ .

DISCUSSION

For convenience the ^{24}Na was obtained from A.E.R.E. in the form of saline made up from irradiated NaCl. Irradiation of the chloride produces a small amount of ^{32}P which might interfere with the interpretation of the experimental results. However, by measuring the decay of a large number of various samples, it was verified that the amounts of long-lived ^{32}P were small enough to be neglected.

 ^{24}Na exchange in nerves in situ

The exchange was more rapid than that described by Manery & Bale (1941) in which the specific activity of a single nerve was only about 50% after

68 min. It can be seen from Fig. 1 that the specific activity of the nerves in the present experiment reached 50% in less than 20 min. It was hoped that *in vivo* experiments would produce more definite evidence concerning the existence of a blood-nerve barrier as suggested by Davson (1951). However, the low accuracy of the experimental points (see Fig. 1 and Table 1) preclude any clear-cut decision on this, though the fact that the experimental points are below a curve constructed from the *in vitro* data on desheathed nerves does support the blood-nerve barrier hypothesis to some extent.

The value of the specific activity (S) at 330 min was 101% (± 15). Thus it can only be claimed from this result that at least 70% of the nerve Na is free to exchange.

It is not possible to calculate a value for the Na efflux from the results illustrated in Fig. 1 because of the large standard errors.

^{24}Na exchange in desheathed nerves *in vitro*

Initial exchange. The first part of the curve of loss of ^{24}Na (Fig. 2) is considered to represent the diffusion of ^{24}Na from the extracellular spaces. The desheathed nerve can be considered as a cylinder, radius a cm, with an initial uniform, extracellular ^{24}Na concentration C'_0 . After a time t sec the average concentration will be

$$\bar{C}' = 4C'_0 \sum_{n=1}^{\infty} \frac{1}{\beta_n^2} e^{-\beta_n^2 D' t / a^2}, \quad (1)$$

where β_n is the n th zero of

$$J_0(\beta) = 0 \quad (\beta_1 = 2.405), \quad (2)$$

and D' is the effective diffusion coefficient of ^{24}Na in the extracellular space (Hill, 1928, p. 71; Keynes, 1954).

After a short time (here about 2 min) only the first term of the series is important and equation (1) reduces to

$$\bar{C}' = \frac{4C'_0}{5.783} e^{-5.783^2 D' t / a^2}. \quad (3)$$

The fast parts of the curves (see, for example, Fig. 2) thus give the quantity $5.783^2 D' / a^2$ and a value of D' , the effective diffusion coefficient of ^{24}Na in the extracellular space can be calculated. The radii, a , which ranged from 420 to 560 μ , were calculated from the weights of the nerves. The mean value of D' was $(1.5 \pm 0.3) \times 10^{-6}$ cm²/sec (range 1.0 to 2.2×10^{-6} cm²/sec). This is to be compared with the self-diffusion coefficient of Na^+ in 0.1 M-NaCl of about 15×10^{-6} cm²/sec. The difference between these two values probably arises from the inexactness of the model chosen, which takes no account of the obstacles to diffusion present in a cylindrical bundle of nerve fibres.

From this value of the effective coefficient of ^{24}Na in the extracellular space of the nerve, it is possible to estimate how far diffusion affects the apparent

odium efflux from the nerve fibres. A theory of this estimation has been given by Keynes (1954, pp. 376-379). By trial and error using Keynes's equations (9), (16) and (18) a mean value for the ratio of apparent efflux to true efflux equal to 0.96 was found.

Slow exchange. The loss of ^{24}Na from nerve fibres into an inactive medium is given by

$$Y = Y_0 e^{-Kt} \quad (\text{Keynes, 1951, p. 14, eqn. (7)}), \quad (4)$$

where K , the rate constant, is given by

$$K = MA/C_i V \quad (\text{Keynes, 1951, p. 14, eqn. (8)}), \quad (5)$$

where M = efflux of Na in mole/cm².sec, V/A = volume/surface area ratio in cm, C_i = intracellular Na concentration in mole/c.c.

The mean value of K ($=K_s$) from measurements on five desheathed nerves was $4.35 \times 10^{-4} \text{ sec}^{-1}$. V/A is to be taken as $2.1 \times 10^{-4} \text{ cm}$ and $C_i = 41 \times 10^{-6} \text{ mole/c.c.}$ (Krnjević, 1955). From these figures $M = 3.75 \times 10^{-12} \text{ mole/cm}^2\text{.sec.}$ This calculation assumes that the whole surface of the nerve fibre is available for Na exchange.) This figure should be increased by about 4% to take account of the fact that exchange takes place into the extracellular fluid from which ^{24}Na has to diffuse; the corrected value of the efflux, M , is $3.9 \times 10^{-12} \text{ mole/cm}^2\text{.sec.}$ Combining the standard errors of K_s , V/A and C_i gives a standard error for M of about 0.6×10^{-12} . It should be remembered that there may be a systematic error of as much as 40% in the chosen value of V/A because of insufficient weighting of the larger fibres. The effect of the wide distribution of fibre size did not, however, reveal itself in any clear deviation from a simple exponential rate of loss of ^{24}Na .

McLennan & Harris (1954) have carried out a somewhat similar experiment using pieces of the cervical vagus nerve of the rabbit. At 0°C they found a value of $K_s = 1.0 \times 10^{-4} \text{ sec}^{-1}$ and at 18°C a value of $K_s = 2.0 \times 10^{-4} \text{ sec}^{-1}$. These values agree rather well with our value of $K_s = 4.35 \times 10^{-4} \text{ sec}^{-1}$, which was obtained at a temperature of 37°C. However, the nerves used by McLennan & Harris were not desheathed, so their figures should perhaps be compared with our figure of $K_s = 0.99 \times 10^{-4}$ (at 37°C), which was obtained from a single experiment with an intact nerve (see below and Fig. 3). No exact comparison can, however, be made between the two experiments, which used different nerves and different methods of loading the nerves with ^{24}Na .

The efflux of Na from the nerve fibres is $3.9 \times 10^{-12} \text{ mole/cm}^2\text{.sec}$ if it is assumed that the whole surface of the axons is uniformly available for the exchange of Na. If only a region near the nodes of Ranvier allows free exchange, then the Na efflux is considerably greater, but in this case it is not immediately obvious that the rate constant (K_s) governing the slow part of the curve of the loss of ^{24}Na can be used to determine the Na efflux, for diffusion along the internodes might be the rate-determining process. In order to investigate

which is the rate-determining step, it is sufficient to calculate the change in concentration of ^{24}Na in a cylinder, length $2l$ cm and cross-section 1 cm^2 , whose surface is impermeable except at the two ends, at which permeation takes place at a rate proportional to the concentration gradient into an outside medium of zero ^{24}Na concentration.

The equation to be solved is thus

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2},$$

with the initial condition:

$$c = C_0 \quad \text{at} \quad t = 0, |x| \leq l$$

and the boundary conditions:

$$\frac{\partial c}{\partial x} = \pm hc \quad \text{at} \quad x = \pm l,$$

where $h = M/DC_i$, and $M = \text{Na efflux across the ends of the cylinder}$, $D = \text{diffusion coefficient of } ^{24}\text{Na in axoplasm}$, $C_i = \text{concentration of Na in axoplasm in moles/c.c.}$

The solution to this problem is (Carslaw & Jaeger, 1947, p. 100)

$$\frac{c}{C_0} = \sum_{n=1}^{\infty} \frac{2h \cos \alpha_n x}{[(h^2 + \alpha_n^2)l + h] \cos \alpha_n l} \exp[-D\alpha_n^2 t],$$

where α_n is a root of $\alpha \tan \alpha l = h$. The rate of loss of ^{24}Na at time t

$$\begin{aligned} &= 2D \left(\frac{\partial c}{\partial x} \right)_{x=-l} \\ &= 2DC_0 \sum_{n=1}^{\infty} \frac{2h^2 l}{h^2 l^2 + \beta_n^2 + hl} \exp[-D\beta_n^2 t/l^2], \end{aligned}$$

where

$$\beta_n = \alpha_n l \text{ is a root of } \beta \tan \beta = hl.$$

The roots of equation (10) have been tabulated for various values of hl (Carslaw & Jaeger, App. iv, table I).

If $hl < 10^{-2}$ then equation (10) reduces to $\beta_1^2 \simeq hl$, $\beta_n \simeq (n-1)\pi$; it is therefore justified to ignore all terms in equation (9) other than the first, so that equation (9) reduces to

$$\begin{aligned} \text{Rate of loss of } ^{24}\text{Na} &= 2DC_0 h e^{-Dhl/l} \\ &= 2M \frac{C_0}{C_i} e^{-Mtl/C_i}. \end{aligned}$$

This is the equation to be expected when the rate-determining step is the efflux of Na across the membrane at the end of the cylinder. Thus the use of K_s to find the efflux from a node of Ranvier is certainly valid if $hl < 10^{-2}$. If $l = 0.075$ cm, $D = 10^{-5}$ cm²/sec and $C_i = 41 \times 10^{-6}$ mole/c.c. this condition corresponds to $M < 0.55 \times 10^{-10}$ mole/cm².sec and $M/|C_i| < 2 \times 10^{-5}$ sec⁻¹.

The experimental value of $M/|C_i|$ (K_s) is about 20 times greater than this limit, corresponding to a value of M of the order of 10^{-9} mole/cm².sec and $hl = 10^{-1}$. Table 3, calculated from Carslaw & Jaeger, App. iv, table I, shows how β_1^2/hl depends on hl in the range (10^{-2} to 1) appropriate to the present problem.

TABLE 3. Values of β_1^2/hl as a function of hl

hl	0.01	0.02	0.04	0.06	0.08	0.10	0.20	0.40	0.60	0.80	1.00
β_1^2/hl	0.998	0.994	0.987	0.980	0.974	0.968	0.937	0.880	0.829	0.782	0.740

It is clear that when $hl \sim 10^{-1}$, i.e. $M \sim 10^{-9}$ mole/cm².sec and $M/|C_i| \sim 4 \times 10^{-4}$ sec⁻¹, β_1^2 is 3–4% less than hl , or the value of the efflux calculated from K_s will be only 3–4% too low because of the effects of internal diffusion. Even if $M/|C_i| \sim 40 \times 10^{-4}$ sec⁻¹, i.e. $hl \sim 1.0$, then the flux will be only about 25% too low.

Thus, at least as far as the present discussion goes, the effects of internal diffusion along the internodes can be neglected. (It can also be shown that for values of $hl < 1$ all terms higher than the first in equation (9) are unimportant.)

An estimate of the magnitude of the Na efflux, assuming that it takes place only in the region of the nodes of Ranvier, can now be made. At the nodes the myelin-free gap is of the order of 0.5μ (Hess & Young, 1952; Stämpfli, 1952). The appropriate average diameter of the axons is 8.4μ as determined from photomicrographs (see above). The approximate relationship between inter-nodal distance (L , mm) and fibre diameter (d , μ) is $L = 0.1d$ (Stämpfli, 1952). Thus the average internodal distance is 1.5 mm if it is assumed that an 8.4μ axon has an external diameter of 15.5μ (Hess & Young, 1952, table 2, p. 309). If, therefore, Na exchange is confined to the myelin-free gap the Na efflux must be $1500/0.5 = 3000$ times greater than 3.9×10^{-12} mole/cm².sec, the value calculated on the assumption that the whole surface of the axon is permeable to Na. On the other hand, a length of the order of 10μ in the region of the nodes may be permeable, for the membrane covering this length has different staining properties from the rest of the axon membrane (Hess & Young, 1952). If this 10μ length is the permeable region then the efflux is $1500/10 = 150$ times greater than 3.9×10^{-12} mole/cm².sec. Both these values of the efflux should perhaps be multiplied by 2, for there is some evidence that the axon is half as wide near a node. Thus the approximate efflux values are:

3.9×10^{-12} mole/cm².sec if the whole axon surface is available for Na exchange,

$(1.2-2.4) \times 10^{-8}$ mole/cm².sec if only the myelin-free gap is available,

$(0.6-1.2) \times 10^{-9}$ mole/cm².sec if a 10μ length in the region of a node is available.

*Significance of the results in relation to the saltatory theory
of nerve conduction*

The value of M calculated on the assumption that the whole nerve fibre takes part in the exchange of Na is of the same order as those values found by previous authors in other excitable tissues; but if it is assumed that exchange of Na can take place only at the nodes, M is of the order of 1000×10^{-12} mole/cm².sec. If the latter is true the value of the resting membrane Na conductance (near a node of Ranvier) is very much greater in the mammalian myelinated nerve than in those described previously (e.g. Hodgkin, 1951).

It would be rather surprising if the mammalian myelinated nerve membrane were to be so different, and it might well be rather difficult to explain the mechanism of such concentrated, intense activity. This might then seem to

argue against the saltatory conduction theory. However, another explanation is possible.

According to Hodgkin (1951, p. 364, eqn. (19)), the Na conductance of a nerve membrane in a steady state is given by

$$G_{\text{Na}} = \frac{F^2 M_{\text{Na}}}{RT}.$$

Substitution of the appropriate values (including $M = 3.9 \times 10^{-12}$ mole/cm²) yields a figure for G_{Na} of about 1.4×10^{-5} mho/cm². In the same article (table 7, p. 394) Hodgkin quotes a figure of 0.16×10^6 ohm.cm² for the resistance of the myelin sheath of a frog nerve fibre recalculated from Huxley and Stämpfli. This is equivalent to a conductance of 6.25×10^{-6} mho/cm². This value may be used here for comparison, it becomes clear that the myelin sheath conductance is probably of the same order of magnitude as G_{Na} , and hence also not very different from the total resting membrane conductance. This suggests that the series resistance provided by the myelin sheath becomes an effective restriction during activity by passively preventing flow of current adequate for local depolarization.

One great advantage of this explanation is its simplicity; there is no need to postulate that the mammalian myelinated axon differs in any fundamental respect from the unmyelinated axon, and no special properties are required of the membrane at the nodes.

Intracellular ²⁴Na

The fraction of ²⁴Na in the slow component of the rate of loss curves is calculated to be $(23.9 \pm 4.9)\%$. It has been shown that the apparent flux of ²⁴Na from the cells is about 96% of the true flux. This difference, which is due to the slowness of the diffusion of ²⁴Na from the extracellular fluid to the bath fluid, implies that the mean specific activity of extracellular Na is about 1/4 of that of intracellular Na. Thus, when t is large, the extracellular ²⁴Na content is about $4\gamma\%$ of the intracellular ²⁴Na content, where γ is the ratio of extracellular to intracellular Na. Since γ is of the order of 4, about 13% of the ²⁴Na activity in the slow component, when t is large, is extracellular. Also, during the first few minutes, the extracellular activity is much greater than the intracellular activity because some of the ²⁴Na initially in the extracellular fluid is still present; high extracellular specific activity at small values of time decreases the rate of loss of intracellular ²⁴Na to values below those obtained by a linear extrapolation of the slow component to zero time.

For these two reasons it is incorrect to say that a linear extrapolation of the slow component to zero time gives a value for the fraction of intracellular ²⁴Na. An exact analysis, to be published elsewhere, shows that the amount by which such a linear extrapolation must be corrected to give the intracellular

depends, essentially, on the ratio K_s/K_F , and that the correction can be quite large. In the present case the average correction is about 25%, the actual figure for the intracellular ^{24}Na being $(18.1 \pm 3.3)\%$ of the total ^{24}Na (3.3 is the S.E. of the mean of five observations).

Change in weight of desheathed nerves in Tyrode

The increase in weight of 35% compares well with the value of 40% found in studies of desheathed bullfrog nerves by Shanes (1953). It is known that all the swelling in desheathed nerves takes place in the interstitial tissue (Lorentz and N6, 1952; Shanes, 1953); therefore the extracellular water content of the desheathed nerve must increase from 43% of the total water (Krnjević, 1955) to about 62% after 3 hr in Tyrode solution. From these figures and the values of the K concentration (32.4 m-mole/kg nerve) and water content (74.8%) of the nerve it can easily be shown that the intracellular K concentration must have been reduced from a normal level of about 180 (Krnjević, 1955) to about 110 m-mole/kg water.

Since there is nothing in the structure of the fibre that could prevent appreciable swelling, it is reasonable to suppose that the axoplasm remains in osmotic equilibrium with the surrounding fluid. Krnjević (1955) mentions evidence that the intracellular fluid is isosmotic with 200 mM-NaCl, despite the fact that the total intracellular concentration (Na + K) is about 223 m-mole/kg water. If this relationship still held for the swollen desheathed nerves then the intracellular (Na + K) concentration would fall to 173 m-mole/kg when the extracellular fluid was Tyrode solution (155 mM). On this assumption the intracellular Na concentration must have increased from the normal value of 11 to about 63 m-mole/kg water.

However this, rather flimsy, argument seems to underestimate the increase in intracellular Na concentration; for the measured Na concentration in the swollen desheathed nerves was 150 m-mole/kg water. The obvious conclusion to derive from this figure is that the intra- and extracellular Na concentrations were both equal to about 150 m-mole/kg water. Thus it seems likely that, during the 3 hr exposure to Tyrode solution, there has been a net increase of Na of between 20 and 110 m-mole/kg water in the nerve axons. This increase would correspond to an average net influx of between 0.4×10^{-12} and 2.2×10^{-12} mole/cm².sec; however it is not known how the net influx varied during the three hours.

^{24}Na exchange in intact nerve in vitro

The slow rate of loss of ^{24}Na from a nerve which had not been adequately desheathed (Fig. 3) agrees with present ideas about the permeability of the nerve sheath (particularly the perineurium). The small fast initial component of the curve presumably represents the diffusion of ^{24}Na adhering to, or actually present within, the superficial layers of the perineurium. The difference between

the rates of escape of ^{24}Na from this and from the other nerves may be compared with that found between intact and desheathed toad nerves in a similar study by Shanes (1954).

^{24}Na space in cats

The rather high mean value for the ^{24}Na space (36.5%) is almost certainly caused by the inclusion in the series of two young kittens; the ^{24}Na spaces of these latter were 43.6 and 58.1%. It is well known that young individuals have a much higher apparent extracellular space than adults of the same species (Manery, 1951). If the values obtained from the six adult cats only were used, the mean is 31.7%; this is a little higher than the values found by other observers in rabbits, dogs and man, quoted by Manery (1951), which is perhaps not surprising since the calculations in the present paper are based upon the activity of the last plasma sample, usually removed after 5–6 hr.

SUMMARY

1. The exchange of Na between cat nerve fibres and the surrounding fluid has been studied using ^{24}Na .

2. The accuracy of the *in situ* experiments was low, but it could be concluded that 50% of the nerve Na was exchanged in less than 20 min and that at least 70% was exchanged in 5–6 hr.

3. The loss of ^{24}Na from desheathed nerves to Tyrode solution *in vitro* was attributed to two processes: a rapid diffusion from the extracellular fluid and a slower loss from the nerve fibres. From the 'fast' diffusion curves a mean value for the effective diffusion coefficient of ^{24}Na in the extracellular spaces equal to $(1.5 \pm 0.3) \times 10^{-6} \text{ cm}^2/\text{sec}$ was calculated. From the 'slow' part of the curves a value for the rate constant (K_s) governing the loss of ^{24}Na from the nerve fibres was deduced; this was $K_s = (4.35 \pm 0.19) \times 10^{-4} \text{ sec}^{-1}$. It was calculated from the parameters of these curves that $(18.1 \pm 3.3)\%$ of the ^{24}Na in the nerve was intracellular. (The quoted errors are standard errors of means of five observations.)

4. From measurements on photomicrographs of lateral popliteal nerve sections the average value of the ratio of the total volume of the nerve fibres to axons to the total surface area was found to be 2.1μ .

5. A value of $3.9 \times 10^{-12} \text{ mole/cm}^2.\text{sec}$ for the efflux (M) of Na from desheathed nerves to Tyrode solution was calculated from the values of K_s and V/A given in paragraphs 3 and 4, taking the intracellular Na concentration to be $41 \times 10^{-6} \text{ mole/c.c.}$ This value of M was obtained on the assumption that the whole surface of the nerve fibre is available for the exchange of Na. If a region near the nodes of Ranvier is available, then $M = (500-25,000) \times 10^{-12} \text{ mole/cm}^2.\text{sec}$.

6. The significance of these values of M for the saltatory theory of nerve conduction is discussed.

7. After the desheathed nerves had soaked for 3 hr in Tyrode solution their weight increased by 35%, the intracellular K concentration decreased from 80 to 110 m-mole/kg water and the intracellular Na concentration increased.
8. The 'Na space' of the cats studied was equal to 36.5% (ml./100 g). S.E. of mean of eight observations = 3.6.)

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Sodium and Potassium Distribution and Transport in the seaweed *Rhodymenia palmata* (L.) Grev.

By

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(Received April 30, 1958)

Introduction

The work described and discussed in this and a subsequent paper (MacRobbie and Dainty 1958) was undertaken to obtain quantitative information on the alkali ion (and chloride) content of and the fluxes between the various compartments of the plant cell and the external medium, under physiological conditions of experiment.

Progress in this field of plant physiology appears to have been slower than in the comparable field of ion transport in animal physiology. This is probably due to the difficulties of interpretation which arise from the morphological complication of the plant systems chosen for study *e.g.* excised roots. In plant tissue is not usually in the same kind of steady state condition as animal tissue. For these reasons it has been possible less often in plant systems to express the results of ion transport studies in fully quantitative terms, an ion flux in pmoles (10^{-12} moles) per sq.cm per second across a well defined boundary separating two phases in which the electrochemical activities of the ions are known.

Marine algae appeared to have many advantages as experimental material for studies on the transport of ions with particular reference to the sodium and potassium distribution and *Rhodymenia palmata*, with its firm flat fronds provided easily handled material. The rates of ion movements could

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ried under physiological conditions by adding tracer quantities of the ion concerned to the normal sea-water medium; it was thus possible to study the material in its normal steady state. However this material has one serious disadvantage — a non-uniform cell population. The fronds of *Rhodomenia* are made up of small cells, about $10\ \mu$ diameter, near the surface and large cells, about $100\ \mu$ diameter in the interior; since the specific rate of exchange may be expected to depend on cell size this makes quantitative flux measurements difficult and uncertain.

Previous work on alkali metal ions in seaweeds has been done by R. Scott (1954), who studied caesium accumulation in *Rhodomenia*, and by G. T. Scott and H. R. Hayward (1953, a, b, c, 1954, 1955, 1957) who studied the sodium and potassium distributions and their metabolic connections in *Ulva lactuca*. Scott found a strong uptake of caesium in light, a process which was inhibited by azide, cyanide and CO_2 -deficiency. G. T. Scott and Hayward found in *Ulva* the usual high potassium, low sodium distribution, and studied the effects of light, iodoacetate, phenyl urethane, dinitro-*o*-cresol, arsenate, and ATP on the ion concentrations. From the results they concluded that the cellular sodium and potassium are regulated by different mechanisms, that the inward potassium pump is closely related to the glycolytic energy-yielding reactions and in particular to those involving phosphoglycerate, and that although the outward sodium pump seems to be coupled to the metabolism of pyruvate, it is not solely dependent on the generation of carbohydrates through photosynthesis, but has a more direct connection with light, perhaps by a redox mechanism of the type proposed by Conway (1953).

In the experiments to be described the rates of exchange of sodium and potassium between the seaweed and the surrounding sea water were measured under a variety of conditions by means of radioactive isotopes. In some respects the experiments are incomplete; the reasons for this will be discussed later. A brief account of some of the early experiments of this study has already been published (Dainty and MacRobbie 1955).

In this paper the terms *extracellular* and *intracellular* are used in the sense of *free* and *non-free space*. Thus the extracellular space would include the spaces between cells, the cell walls and any part of the cytoplasm open to free diffusion. Intracellular space would be the rest of the cytoplasm plus the vacuoles.

Methods

Plants were collected from North Berwick, Scotland, and were stored at 8°C for a limited time (up to a month) before use in aerated tanks of sea water from their natural habitat and illuminated for about 8 hours per day. Most of the experiments

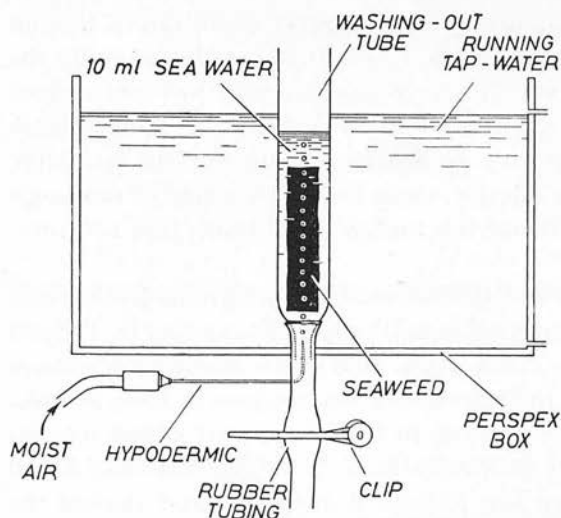


Figure 1. Diagram of the apparatus used in the washing-out experiment.

were done on rectangular strips cut from the fronds, 4 cm. by 1.2 cm. and weighed about 0.26 g. (wet weight); sometimes sets of six discs 1 cm. in diameter were used.

The isotopes used were ^{24}Na and ^{42}K , each of which was obtained from A.E. Harwell, as the spec.-pure carbonate. After conversion to the chloride by titration with N-HCl , the labelled salt was added to a suitable volume of sea water. This changed the concentration in the sea water by about 3% for Na and about 1% for K; an attempt was made to minimise the disturbance these changes create in the dynamic equilibrium of the cells by soaking the seaweed for 12–24 hours before the start of an experiment in an inactive modified sea water of the same chemical composition as the appropriate active sea water to be used. The radioactivity of the active sea water at the beginning of each experiment was about 20 $\mu\text{C}/\text{ml}$. for ^{24}Na -labelled sea water and about 0.1 $\mu\text{C}/\text{ml}$. for ^{42}K -labelled sea water. For this represents an initial dose rate of about 20 rad/hour or a total dose of about 200 rad per experiment in the worst case; this might have decreased the effect somewhat (Barber, Neary and Russell 1957), but we had no indications from our experiments themselves that it did and we shall assume that there was no radioactive effect on the fluxes.

The seaweed was placed in aerated, stirred, radioactive sea water for some time, usually overnight, until a suitable amount of the intracellular K or Na had been changed with the radioactive isotope in the sea water. Usually both the amount of activity taken up in a given time and, by a 'washing-out' experiment, the subsequent loss of this labelled ion to an inactive medium were measured. In a washing-out experiment the strip of seaweed was transferred, after blotting lightly, from the active medium to 10 ml. of inactive modified sea water in a washing-out tube. This is shown in Figure 1 and consisted of a 10 cm. long pyrex tube, of internal diameter 1.5 cm. drawn down at one end to an external diameter of about 0.5 cm. which was closed with a short length of rubber tubing and a spring clip. The 10 ml. of inactive sea water was stirred and oxygenated by moist air introduced by a

a fine hypodermic needle thrust through the wall of the rubber tubing. At given times the sample was run out into a specimen tube by releasing the spring clip, and fresh 10 ml. of inactive sea water poured into the tube. Thus each 10 ml. sample contained the ^{24}Na or ^{42}K lost from the seaweed in a specified time interval, and measuring the radioactivity of each sample the rate of loss of ^{24}Na or ^{42}K was determined as a function of time. At the end of each experiment the amount of radioactivity left in the seaweed was found by wet-ashing it in 10 ml. of a 1:1 mixture of conc. H_2SO_4 and conc. HNO_3 and counting this sample.

The activities of the 10 ml. samples were determined by counting in M6 liquid counters using standard probe units, scalars and power units. In addition to the usual corrections for background, dead-time and radioactive decay, corrections were also applied for differences in self-absorption in liquids of different densities and for the amount of radioactivity left in the washing-out tube when a sample was run out. During the experiments the seaweed and the bathing solutions were maintained at a temperature of 8°C and in general two experiments, a 'light' experiment ('daylight' fluorescent lighting, 2,000 metre candles) and a 'dark' experiment (blackpainted covered tube), were run in parallel.

The amounts of Na and K in the seaweeds were determined by means of an EEL flame photometer. The material was first soaked in isotonic sucrose solution for 10 minutes to remove extracellular Na and K, then oven-dried for 24 hours at 110°C and finally dry-ashed in a platinum crucible in a muffle furnace for 24 hours at 600°C . The ash was dissolved in the minimum of dilute HNO_3 and this solution was suitably diluted for flame photometry. The intracellular chloride content was also measured on this solution by electrometric titration with AgNO_3 .

Results

Percentage of water in the seaweed

Comparison of the wet weight with the weight after oven-drying at 110°C for 24 hours gave the percentage of water in the seaweed. The water content was 80 ± 1 g. water/100 g. wet weight (10). [Such results will be quoted in the form: mean \pm S.E. of mean (number of results on which mean is based).]

Chemical determinations

These results represent the total Na, K and Cl in the seaweed after 10 minutes soaking in isotonic sucrose solution. It was assumed that ions remaining after this treatment were intracellular although the radioactive experiments later showed that these ions had a non-uniform exchange rate. The interpretation of this discrepancy will be discussed at a later stage.

The values found were: K, 415 ± 20 $\mu\text{moles/g. wet weight}$ (8); Na, 16.5 ± 1.5 $\mu\text{moles/g. wet weight}$ (8); Cl, 300 ± 20 $\mu\text{moles/g. wet weight}$ (6). The chloride value is likely to be an underestimate because of the possibility of loss during the ashing.

Radioactive measurements

Theory. — The equations governing the exchange of radioactive ions between a cell and the surrounding solution have been given by Ussing (1948) and others. A brief statement is necessary here before the results of measurements are given.

If a cell containing no radioactive ions is placed in a large volume of solution in which the total external concentration of the ion species in question is C_o , the external concentration of labelled ions is C_o^* , then the initial rate of entry of labelled ions into the cell is given by:

$$\frac{dc_i^*}{dt} = M_{in} \frac{A}{V} \frac{C_o^*}{C_o}$$

where A is the surface area, V is the volume, C_i^* is the internal concentration of labelled ions and M_{in} is the influx of the ion in moles/cm.²sec. After some time when radioactive ions are crossing the cell boundary in both directions, the rate of change of internal activity is given by:

$$\frac{dc_i^*}{dt} = M_{in} \frac{A}{V} \frac{C_o^*}{C_o} - M_{out} \frac{A}{V} \frac{C_i^*}{C_i}$$

where C_i is the internal concentration and M_{out} is the efflux of the ion in moles/cm.²sec. In a washing-out experiment, in which the radioactive ion is not allowed to accumulate in the external medium, C_o^* may be taken as zero at all times and the solution of equation (2) is thus:

$$C_i^* = C_{i0}^* \exp \left(- \frac{M_{out}}{C_i} \frac{A}{V} \cdot t \right)$$

where C_{i0}^* is the initial concentration of labelled ion in the cell. Thus if $\ln C_i^*$ is plotted against time the graph will be a straight line of intercept $\ln C_{i0}^*$ and slope $-M_{out} A/VC_i$. The slope, which we shall call k , allows calculation of the efflux provided the internal ion concentration and the surface/volume ratio of the cell are known. The influx may be calculated from the uptake over a period short enough for the internal radioactive ion concentration to be considered negligible, by use of equation (1).

With a tissue such as *Rhodymenia*, the exchange of ions is, in principle, more complicated than the above equations suggest. The exchange is at least a two-component process: a rapid exchange by diffusion from the extracellular spaces and a much slower exchange across the protoplasmic membrane. Mathematical solutions of this two-component process have been given for special cases, by Harris and Burn (1949), and Dainty and MacRobbie (unpublished). These show that, provided the rates of the two processes are sufficiently different, their separate contributions may be calculated and added to give the amount of radioactivity against time (in a washing-out experiment this reduces to the sum of an exponential, whose time constant is governed

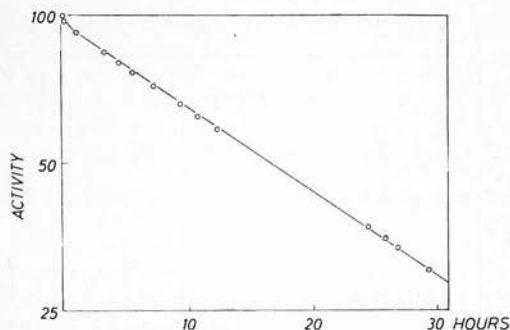


Figure 2. Exchange of radioactive potassium; activity left in the seaweed plotted logarithmically against time.

rate of exchange across the protoplasmic membrane, and a diffusion curve, governed by the rate of diffusion from the extracellular space. The conditions for adequate separation of these two components are amply fulfilled in bathymenia and one would therefore expect the semi-log plot of a washing-out experiment to reduce to the sum of two straight lines (since a diffusion curve is exponential after a brief initial period), whose slopes are related to the rates of the diffusion and exchange processes and whose intercepts are related to the amounts of extra- and intracellular ion.

These two fractions were in fact found, but the intracellular component appeared to have a non-uniform rate of exchange and this was more marked for Na than for K.

The interpretation of the experimental curves for Na and K will be considered separately.

Potassium. — A typical 'washing-out' curve of the amount of radioactive potassium left in the seaweed plotted logarithmically against time is shown in Figure 2. This strip had taken up radioactive potassium for 40 hr. and it can be seen that after this time over 90 % of the radioactivity was in the slowly exchanging fraction and therefore probably intracellular.

Intracellular potassium. The intercept of the 'slow' straight line on the axis $t=0$ represents — so we shall argue — the initial intracellular radioactivity; the percentage of exchange during the time of uptake may be calculated from the rate of exchange of this slow fraction and hence the amount of exchangeable potassium in the cells may be obtained. The uptake times were such that 40—95 % of the total intracellular potassium was labelled at the start of a washing-out experiment. The value found for intracellular potassium was 315 ± 15 μ moles K/g wet weight (8).

Extracellular space. The contribution of the intracellular fraction to the total radioactivity in the initial rapid exchange is estimated from the slow component and is then subtracted from the total; the remainder is replotted,

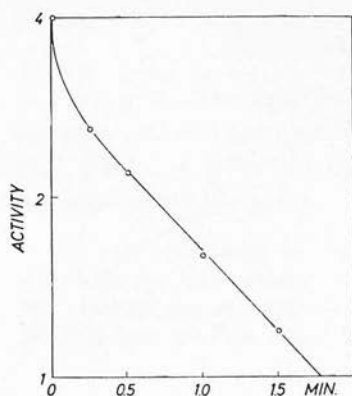


Figure 3. Exchange of extracellular potassium; cellular activity plotted logarithmically against

again logarithmically, against time to find the amount of extracellular potassium and its rate of exchange. A typical result is shown in Figure 3; this is a diffusion curve in which the intercept of the straight line part of the curve should have the value $8/\pi^2$ times the amount of K in the extracellular space and its slope should have the value $\pi^2 D'/l^2$, where D' is the apparent diffusion coefficient in the extracellular space and l is the thickness of the seaweed strip. It was assumed that the concentrations in the extracellular space were the same as those in the external sea water and hence the volume of the extracellular space was calculated. This volume was found to be 0.237 ± 0.008 g. wet weight (14). From the slope D' was found to be $(3.6 \pm 0.4) \cdot 10^{-6} \text{ cm}^2/\text{sec.}$ The hindrance to free diffusion imposed by the presence of cells in the tissue is reflected in the difference between this value and the value $16 \times 10^{-6} \text{ cm}^2/\text{sec.}$ — the probable diffusion coefficient of K in sea water. It should be stated here that the values for the extracellular volume calculated from sodium and potassium experiments were in agreement.

Intracellular concentration. From the amount of water in the seaweed and the volume of the extracellular space, the amount of intracellular water can be calculated. Thus in 1 g. of tissue (wet weight) the weight of total water is 0.80 ± 0.01 g., of extracellular water 0.234 ± 0.008 g., and hence the weight of intracellular water is 0.566 ± 0.015 g. Using this value and the value of 3.6 ± 0.4 $\mu\text{moles/g.}$ wet weight for the intracellular K, we find the average intracellular K concentration in the cell water to be 560 ± 40 $\mu\text{moles K/g.}$ of water.

Rate constants for K exchange across the protoplasmic membrane. Although the rate constant k ($=M_{\text{out}}A/VC_i$) for the exchange across the protoplasmic membrane may be estimated from the slope of the slow part of the washing-out curve, a more accurate value, given our experimental method

Table 1. Rate constants ($10^2 k \text{ hr}^{-1}$) for potassium exchange across the cell 'membrane'.

Conditions	Over $t = 0$ to 1 hr		Over $t = 2$ to 12–40 hr	
	Range	Mean	Range	Mean
Light (2000 metrecandles)	5.0 — 12.0	7.0 ± 1.2 (6)	3.8 — 5.6	4.8 ± 0.3 (5)
Dark	0.66 — 2.16	1.3 ± 0.2 (7)	0.38 — 0.68	0.48 ± 0.06 (5)

may be found by calculating the specific rate of exchange for each time interval. This is $\frac{1}{c_i^*} \frac{dc_i^*}{dt}$ or $\frac{\text{counts/min lost per unit time}}{\text{counts/min present in seaweed}}$. By this means any apparent variation of k with time is clearly seen and it is possible to compare closely the behaviour of the intracellular fraction with that of a simple compartment whose ions exchange at a single uniform rate. In fact some deviation from this simple system was found and the specific rate of exchange of the intracellular potassium was higher during the first hour's washing-out than the steady value assumed in the later part of the experiment. The values found for k are summarised in Table 1; the values over the first hour were obtained from the slope of the curve during that period, but the final steady rate constant for each strip of seaweed was calculated as the mean of the values during a number of time intervals, usually from 5 to 10 points over a period of 5–12 hours at least. For individual strips of seaweed the standard error of the mean calculated from the values in these time intervals was never more than 12%, and was usually 1–5%. The range of k values for different strips is given, together with their mean. Rates of exchange in both light and dark are quoted since the effect of illumination is very marked.

Since the rate constants for different strips of seaweed show considerable spread the most reliable demonstration of the effects of illumination, or of any other specific factor, is the change in rate of exchange of a given piece of seaweed on the appropriate change in conditions. That the efflux is much faster in light than in dark is clearly seen both from the values of Table 1 and from the graph of Figure 4. The rate constants for this latter experiment are given in Table 2, together with those for the parallel experiment in which the light-dark periods were interchanged.

It was noticed that when the change to light was made after a prolonged period of darkness k rose gradually to a maximum before falling slightly to a steady value, whereas after only a short period of darkness the full 'light' value of k was attained immediately on re-illumination. Similar differences were seen in the effects of the change from light to dark; after a prolonged period of light the rate of exchange did not fall immediately to the typical 'dark' value but remained rather higher for at least four hours, whereas with

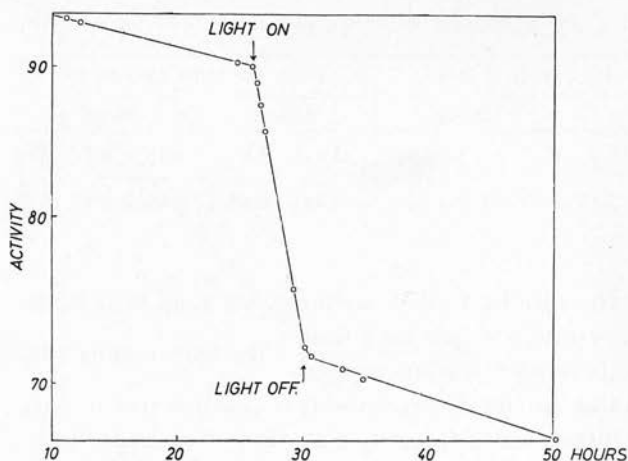


Figure 4. Exchange of cellular potassium in light and dark.

a dark-adapted seaweed the normal 'dark' value was reached within 10 hours of the return to dark after a short period in the light.

Potassium fluxes. The efflux of potassium from the cells may be calculated from the results of the washing-out experiments from the formula:

$$M_{\text{out}} = k \frac{V}{A} C_i$$

There are serious difficulties in applying this formula to the results described above, all arising from the uncertainties inherent in measurements on a non-uniform cell population. The volume/surface ratio of a spherical cell has the value $d/6$, where d is the cell diameter, and hence varies directly with the cell size. Both the other quantities, k and C_i , may be expected to vary with cell size. The most probable explanation of the variation of k over the course of a washing-out experiment is that the small cells of the population exchange potassium more rapidly than the large ones and therefore contribute a disproportionate amount to the early part of the exchange; the amount of potassium in the 'small cell' fraction is not large — only a few percent of the total slow fraction.

Table 2. Rate constants ($10^2 k \text{ hr}^{-1}$) during light-dark periods for two strips of seaweed, A and B.

Time	A		B	
	Light	Dark	Light	Dark
From 2 to 26 hr. ...	4.9 ± 0.3 (7)	0.38 ± 0.02 (9)	5.58 ± 0.05 (13)	0.98 ± 0.05 (6)
From 26 to 30 hr. ...		0.44 ± 0.05 (4)	5.6 ± 0.1 (6)	

An estimate of the efflux from the large cells may be obtained from the value for the period 2—12 hr., V/A for the large cells, the total exchangeable potassium in the slow fraction and an estimate from histological sections of the volume of tissue occupied by the large cells. This gives an efflux of 8 pmoles K/cm.²sec. in light and 1.8 pmoles K/cm.²sec. in the dark. These estimates are probably reliable to within a factor three, although the light/dark ratio is much more reliable.

It is not possible to calculate an influx figure comparable with the efflux given above. The influx into the cells must be calculated from the intracellular uptake of labelled ion in a time, short compared with the half-time for exchange across the protoplasmic membrane, during which the internal concentration of labelled ions remains small enough for their outward movement to be neglected. The rate of entry of labelled ion is then given by equation (1) and the influx takes the form:

$$M_{in} = \frac{1}{A} \frac{C_o}{C_o^*} \frac{d}{dt} (VC_i^*) = \frac{C_o}{C_o^*} \frac{Q^*}{At} \quad (5)$$

where Q^* is the amount of labelled ion taken up in time t . However at short uptake times the effects of the small, more rapidly exchanging cells become more important, and the compartment involved in the exchange is ill-defined. Over periods of 1—2 hr., uptakes of 8—11 μ moles K/g. wet weight/hr. in light and 1.8—4.0 μ moles K/g. wet weight/hr. in the dark were found. The figures for the outward movement of potassium most nearly comparable with these are obtained by multiplying the mean rate constant over the first hour of a washing-out experiment by the amount of intracellular potassium; this gives outward movements of 19 μ moles K/g. wet weight/hr. in light and 1.8 μ moles K/g. wet weight/hr. in the dark. Thus the movements inwards and outwards are of the same order of magnitude, but since the method of calculating the outward movement is open to a number of objections if the two figures are to be compared, it is not justifiable to draw any conclusions from the differences between them.

Effects of dinitrophenol. Two experiments involving six strips of seaweed were done on the effects of DNP at a concentration of 4×10^{-4} M. The efflux experiments were done by allowing the seaweed to take up K under normal conditions and then following the rate of loss of activity during three successive 4 hr. periods in sea water, sea water + DNP, sea water. In light the efflux was reduced in DNP by a factor of 0.77 ± 0.02 . In the only experiment done in the dark there was a reduction of the efflux by a factor of 0.83. The influx in DNP-sea water was also compared with that in normal sea water. In light the influx in DNP was reduced by a factor of 0.81; the effect on the influx in the dark was not measured.

Table 3. *Effects of added glutamate on potassium fluxes in the dark.*

Glutamate concentration mM	Ratio: <i>efflux in sea water + glutamate</i> <i>efflux in sea water</i>	Ratio: <i>influx in sea water + glutamate</i> <i>influx in sea water</i>
10	2.0 ± 0.2	not measured
25	2.3 ± 0.2	2.4
50	3.4 ± 0.1	not measured

Effects of glutamate. Solid sodium glutamate was added to modified water to give glutamate concentrations of 10, 25 or 50 mM. The pH of such solutions was adjusted to the normal sea water pH. The experiments were always done on two strips of seaweed cut from the same frond; 'normal' and 'glutamate' treatments were alternated. The results showed the addition of glutamate had no effect on the efflux of potassium in the light but it had marked effects on the efflux (and influx) in the dark. The results are collected in Table 3.

Sodium. — The treatment of the experimental results for sodium is similar to that for potassium; the washing-out curves are basically similar but differ in the relative amounts of the various fractions. As for potassium the extracellular fraction was found by subtraction of the estimated intracellular radioactivity from the total activity, but for sodium about 90 % of the Na was extracellular. (See Figures 5, 6, 7.)

Extracellular space. The loss of sodium from the extracellular space was found from a diffusion curve and the amount of sodium and the apparent diffusion coefficient of sodium in this fraction were found from the straight line on the log. plot of the latter part of this diffusion curve (Figure 5). It was assumed that the sodium concentration in the extracellular space was the same as in the external sea water and the volume of the space was calculated. A seasonal variation in the volume of the extracellular space and the apparent diffusion coefficient was found; the extracellular volume of 'summer' seaweed was 0.20 ± 0.01 ml./g. wet weight (7) and of 'autumn' seaweed 0.008 ml./g. wet weight (10); the corresponding apparent diffusion coefficients were $(4.7 \pm 0.3) \times 10^{-6}$ cm.²/sec. and $(3.3 \pm 0.1) \times 10^{-6}$ cm.²/sec. The second figure for the extracellular volume agrees exactly with the value quoted already for potassium experiments on the same type of seaweed. The exactness of the agreement is of course fortuitous, but it does support the assumptions on which the calculation of extracellular volume is based. The apparent diffusion coefficients for Na in the extracellular space are less than the value in sea water (10×10^{-6} cm.²/sec.), thus again illustrating the hindrance to diffusion of the presence of cells.

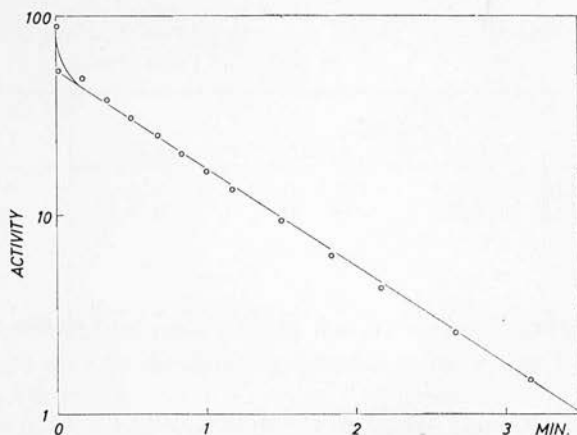


Figure 5. Exchange of extracellular sodium.

Intracellular sodium. The total amount of intracellular activity may be found from the intercept at $t=0$ of the slow fraction in the semi-log. plot of washing-out experiment (see Figure 7). The mean value for the total intracellular sodium, from 9 experiments, was 14 ± 1 μ moles Na/g. wet weight (9).

Since the non-uniformity of the exchange rate was so marked, a further analysis of the washing-out curve allowed a better characterisation of the intracellular sodium and its exchange rate. As in the potassium experiments the rate constant k fell to a final steady value after about 2 hours of washing-out, but the amount of sodium in this final slow exchange was appreciably less than the total intracellular sodium. It was possible to find a second intracellular fraction with a more rapid rate of exchange by subtracting the con-

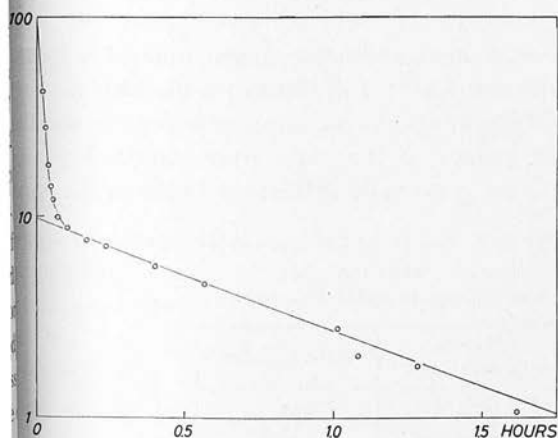


Figure 6. Exchange of sodium in the fast intracellular fraction II.

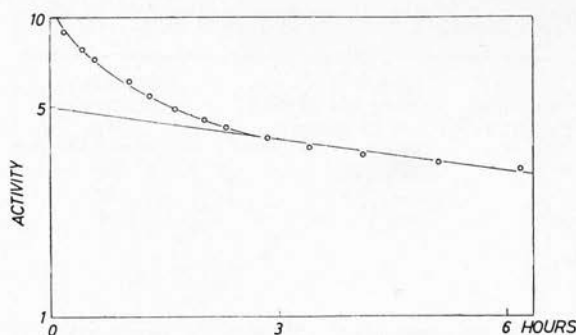


Figure 7. *Exchange of intracellular sodium; the straight line represents the exchange with slow intracellular fraction I.*

tribution of the slowest fraction from the total intracellular activity and plotting the remainder logarithmically against time. An example of the contractions of the three fractions, extracellular Na and the two intracellular fractions, is shown in Figures 5, 6, 7. The mean value of the amount of Na in the slow intracellular fraction was 9.0 ± 1.3 $\mu\text{moles Na/g. wet weight}$ and in the fast intracellular fraction — 5.0 ± 0.5 $\mu\text{moles Na/g. wet weight}$ (After the usual uptake times only the slowest fraction was incompletely labelled and allowance for this was made in the calculation of the total activity from the amounts of labelled ion.)

The mean concentration of sodium in cell water was found from the ratio of intracellular sodium and the total intracellular water to be 25 ± 3 $\mu\text{moles/l. cell water}$. The intra- and extracellular Na and K concentrations, from radioactivity measurements, are collected in Table 4.

Rate constants for sodium exchange across the cell membrane. The effective rate constants for Na exchange in light and in dark are summarised in Table 5 for both fraction I, the slowly exchanging cells, and fraction II, the more rapidly exchanging cells.

The ratios of the mean value of k in light to the mean value of k in dark are 1.5 ± 0.3 in the slow cells and 1.8 ± 0.2 in the fast cells. Experiments in which the effect of light and dark was especially studied gave similar values; in three experiments six values of the ratio were obtained with a mean was 1.5 ± 0.1 (6); there was no systematic difference between the

Table 4. *Ion concentrations in $\mu\text{moles/g. water}$.*

Ion	Mean intracellular	Extracellular
Na	25 ± 3	467
K	560 ± 40	11.0

Table 5. *Efflux rate constants ($10^3 k \text{ hr}^{-1}$) for sodium exchange across the protoplasmic 'membrane'.*

Conditions	I		II	
	Range	Mean	Range	Mean
Light	24 — 39	30 ± 4 (4)	76 — 180	130 ± 10 (8)
Dark	15 — 26	19 ± 2 (4)	59 — 99	74 ± 6 (8)

and the fast cells. These results may be combined in the statement that the sodium efflux is greater in light than in the dark by a factor in the range 1.3 to 1.8, with a mean value of 1.5 ± 0.1 (8).

Sodium fluxes. Since the slow and fast intracellular fractions were separable, the total inward and outward movements in the cells of 1 g. of tissue may be found with reasonable accuracy. The outward movement (in $\mu\text{moles/g. wet weight/hr.}$) from the cells, M'_{out} , is given by:

$$M'_{\text{out}} = M'_{\text{I out}} + M'_{\text{II out}} = k_1 Q_1 + k_2 Q_2 \quad (6)$$

where k_1 , k_2 and Q_1 , Q_2 are the rate constants and the amounts of Na in the respective intracellular fractions. The values of M'_{out} and its component parts, in light and in dark, are given in Table 6. Also given in the same table are the inward sodium movements, M'_{in} , in light and in dark, calculated from the amount of labelled Na taken up during a short time. Periods of 10—30 min. were used, but since in light there was appreciable exchange in this period, the equation

$$M'_{\text{in}} = Q^* \frac{kt}{1 - e^{-kt}} \quad (7)$$

was used to calculate the inward movement. (Q^* is the uptake per unit time and k is the rate constant for washing-out determined for the same strip over time t , the uptake time.) From these figures the assumption that the tissue is in a steady state appears to be justified.

Table 6. *Sodium movements, M' , in $\mu\text{moles Na/g. wet weight per hour}$, in the slow cells (I), the fast cells (II), and the whole tissue.*

Movements	Light	Dark
$M'_{\text{I out}}$	2.4 ± 0.4	1.6 ± 0.3
$M'_{\text{II out}}$	7.0 ± 1.1	4.9 ± 1.0
$M'_{\text{out}} = M'_{\text{I out}} + M'_{\text{II out}}$	9.4 ± 1.2	6.5 ± 1.0
M'_{in}	10.1 ± 0.5 (3)	6.8 ± 0.5 (6)

Table 7. *Approximate efflux from large cells in pmoles/cm.² sec.*

Conditions	Na	K
Light	1.8	18
Dark	1.2	1.8

If the slow fraction is attributed to the large cells of the population, it seems reasonable, approximate values for the Na efflux may be calculated from these figures as was done for the K efflux. These are given in Table 7 together with the potassium figures already given; they are both uncertain by about a factor three.

The effects of DNP and glutamate on the sodium movements in *Rhododymenia* have not been studied.

Discussion

While this work allows some conclusions to be drawn on the sodium and potassium distribution and its maintenance in *Rhododymenia*, accurate figures cannot be quoted, nor can any really reliable assessment be made of the contributions of different cellular phases to the ion distribution and transport. It is for these reasons that in some respects the work was not complete and many interesting and important problems were not followed up.

The amounts and rates of the very fast initial exchange are consistent with a loss by diffusion from the extracellular spaces. Both sodium and potassium diffusion coefficients in the extracellular space are less than the probable values in sea water, as is expected in a tissue where the presence of relatively impermeable cells hinders free diffusion both by reducing the effective space for diffusion and by lengthening the diffusion path. The ratio of the potassium to the sodium diffusion coefficient is also reduced, but we cannot suggest why. The volume of the extracellular space was calculated on the assumption that it is filled with sea water and the agreement between the sodium-space and the potassium-space supports this assumption. The extracellular space should have been determined for molecules other than monovalent cations, but this was not done. The presence of a very high immobile ion concentration in the free space would raise the mobile cation concentration and lead to an overestimate of the extracellular volume by this method. It is thought that any serious error arising from neglect of this possibility is unlikely. There is known to be a relatively large extracellular space in this tissue in which the ion concentrations must have the external values, and

mobile anion concentrations in any additional Donnan phase would need be exceedingly high compared with an already high external concentration change seriously the total amount of ion in the combination of the two phases.

The interpretation of the intracellular fractions and their heterogeneous rates of exchange presents difficulties. As has been stressed already it is not to be expected that the exchange in a non-uniform cell population should be controlled by a single rate constant. In addition each cell probably contains at least two phases — 'protoplasmic non-free space' and vacuole (MacRobbie and Dainty 1958) — in which the ions have relatively slow rates of exchange. We think it reasonable, however, to assume that the final steady value of the rate constant in a washing-out experiment represents loss from the vacuoles of the large cells of the population, those in the centre of the flat thallus, and that the initial faster rate represents loss from the smaller cells (and, possibly, from the protoplasmic non-free space of all the cells). This implies that the vacuoles of the large cells would contain about 310 μ moles K and 9 μ moles Na, per g. tissue wet weight. (Here we are taking the intracellular potassium determined from the radioactive measurements; this is exchangeable potassium and presumably exists as free potassium ions in solution in the vacuole. The electrochemical potential will be determined by this free potassium. The difference between the chemical and radioactive measurements suggests that about 100 μ moles K/g. tissue wet weight is bound in an unexchangeable form.) From tissue sections it was estimated that about $\frac{2}{3}$ of the intracellular water in the large cells — say 0.4 ml./g. tissue wet weight — and hence the intracellular K and Na concentrations will be about 800 mM and 25 mM respectively. The principal balancing anion is presumably chloride for chemical determinations show that the intracellular chloride is about 75 per cent or more, on a molar basis, of the intracellular potassium.

After earlier failures during the course of this work it has recently been found possible to insert KCl microelectrodes into the large cells and thus measure the potential difference between vacuole and external sea water (Johnston and Williams, in this laboratory, private communication). Measurements on twelve cells gave a fairly constant value of -65 mV (vacuole negative) and this enables us to discuss which ions are actively transported. For purely passive exchange between two phases the following relation between concentration (strictly activity) and equilibrium potential difference must hold:

$$E = \frac{RT}{zF} \ln \frac{C_o}{C_i} = \frac{58}{z} \log_{10} \frac{C_o}{C_i} \text{ mV} \quad (8)$$

where E is the potential difference between inside (i) and outside (o) and z the charge on the ion in electron charges. The equilibrium potential dif-

ferences for K, Na and Cl calculated from this equation are approximately -110 mV, $+75$ mV and zero respectively. Comparison with the observed potential difference of -65 mV shows that the electrochemical potentials for K and Cl are higher inside than outside but that of Na is much lower inside than outside. If we assume that the cell membranes are permeable to Cl, the figures suggest that K and Cl are actively transported *into* the cell (vacuole) and Na is actively transported *out*. As with animal cells (Harris 1956) for which both concentration and potential gradients are directed inwards, the farthest from electrochemical equilibrium but, unlike most animal cells, must be 'pumped' into these plant cells. This latter point is discussed in detail in a later paper (MacRobbie and Dainty 1958), where more quantitative information is available on another cell. K is fairly close to electrochemical equilibrium and may be closer than the figures suggest, for a fairly large, tough, microelectrode, of tip diameter $2-3\ \mu$, had to be used to penetrate the cell walls; this may damage and somewhat depolarise the cell and would result in a lower potential reading, though there was little sign of a potential of -65 mV being maintained for some hours.

The K efflux, in light, from the large cells is of the same order of magnitude as in squid nerve and frog muscle (Harris 1956); the Na efflux, however, is about ten times smaller. If, in the light, the influxes are equal to the corresponding effluxes and if both the K and Na influxes are purely passive (though there is good reason to doubt this), then the cell 'membrane' is about 400 times more permeable to potassium than to sodium. This is a much higher permeability ratio than the usual 20–100 figure in other cells (Conway 1950).

The apparent energy requirement for extruding Na from the cell, assuming that all the Na efflux is due to active transport, is about 5×10^{-7} cal./hour per large cell or, if $1/10$ of the cell mass is protoplasm, 0.1 cal./hour/g. protoplasm (see Keynes and Maisel 1953, for method of calculating energy requirements). This value is quite high and to it must be added an unknown amount for pumping chloride, and perhaps potassium, into the cell; it therefore seems probable that part of the fluxes may be due to exchange diffusion (Ussing 1947) which would reduce the energy requirement.

The effect of light is to increase the rates of exchange of both K and Na but it is most pronounced on the rate of K exchange in the large cells, where the efflux in light is about eight times the 'dark' value and the influx is also greater. It was not possible to decide whether the two fluxes changed in exactly the same proportion; probably (see Scott and Hayward 1955) the Na efflux is greater than the influx in the dark, giving a net loss of potassium. Though there may be some active transport of potassium inwards, the Na fluxes are probably mostly passive or due to exchange diffusion. If the Na fluxes are purely passive, *i.e.* K ions are diffusing across the membrane as ion

light must directly affect the permeability of the cell membrane, possibly widening water-filled pores, though not enough to affect the sodium fluxes appreciably. It is more attractive to attribute the K fluxes to exchange fusion and the effect of light to a great increase in the supply of K carriers. The time course of the light-dark effect is compatible with the building up of carriers during photosynthesis in the light and their removal by respiration in the dark.

In view of the fact that sodium must certainly be actively transported out of the cell the light-dark effect on the sodium fluxes, and particularly on the Na flux, is surprisingly small. This suggests that Na transport is linked much more closely with respiration than with photosynthesis. It is possible to speculate about these links but this would be unrewarding until the necessary further experiments on the effect of metabolic inhibitors have been carried out. Their effects on Na transport have not been studied and the few experiments on the effect of DNP on the K fluxes are not very informative; the effect was small.

The effects of glutamate on the K fluxes in the dark are most readily explained by supposing that glutamate stimulates the formation of carrier molecules. This need not imply that glutamate itself or a close derivative is the carrier; glutamate has extensive metabolic connections and may be transformed into a variety of substances occupying key positions in the cell metabolic schemes. The effect of other amino-acids and of substances related to other aspects of glutamate metabolism might suggest in what capacity glutamate is acting.

The discussion so far has been concerned with the slowest exchanging fraction containing the bulk of the intracellular K and 60 % of the intracellular Na, which we have reasonably associated with the vacuoles of the large cells. The faster exchanging intracellular fraction is probably of a more heterogeneous origin and we consider that speculation about its characteristic properties — Na/K ratio, effect of light, etc. — is unwarranted in view of the uncertainty of its morphological position.

The work discussed in this paper goes some way towards fulfilling the purpose with which it was undertaken, which was to obtain quantitative information on the ion content of the various compartments of the tissue and the ion fluxes between them and the external medium. But as long as it is necessary to allow for the effects of a non-uniform cell population in interpreting the results it is not possible to separate the behaviour of different subcellular compartments; the ionic relations within the cell involving the protoplasm, cytoplasmic inclusions and vacuole, though of fundamental importance cannot be distinguished from the effects of variations in cell size. It was there-

fore thought that work on a tissue morphologically less complicated be of greater value and attention was transferred to the giant Characean this work is reported in subsequent papers.

Summary

The sodium and potassium distribution and transport in *Rhodomenia palmata* have been studied using ^{24}Na and ^{42}K . Chemical measurements show that the seaweed contained 0.80 ± 0.01 g. water, 415 ± 20 μmoles intracellular potassium, 16.5 ± 1.5 μmoles intracellular sodium and 300 ± 20 μmoles intracellular chloride, all per g. tissue wet weight. Radioactive determinations of intracellular exchangeable ion contents gave 315 ± 15 μmoles K and 16.5 ± 1.5 μmoles Na per g. tissue wet weight. The tissue contained an extracellular space, filled with sea water and open to free diffusion, of amount 0.24 ml./g. tissue wet weight.

Kinetic studies of the exchange of K and Na between the tissue and external sea water indicated that there was more than one intracellular compartment in the tissue. However most of the potassium and 60 % of the sodium showed a uniform slow exchange rate and this compartment was assigned to the large central cells of the fronds which contain about 80 % of the intracellular water. There was a marked light-dark effect on the potassium fluxes, but only a small effect on the sodium fluxes. Estimates of fluxes are given and the effects of DNP and glutamate on the potassium fluxes were studied.

The results are discussed in terms of current ion transport theories and the difficulties of working with non-uniform cell populations are stressed.

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ION TRANSPORT IN NITELLOPSIS OBTUSA

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ABSTRACT

The distribution and rates of exchange of the ions sodium, potassium, and chloride in single internodal cells of the ecorticate characean, *Nitellopsis obtusa*, have been studied.

In tracer experiments three kinetic compartments were found, the outermost "free space" of the cell, a compartment we have called "protoplasmic non-free space", and the cell sap.

The concentrations in the vacuole were 54 mM Na⁺, 113 mM K⁺, and 206 mM Cl⁻. The steady state fluxes across the vacuolar membrane were 0.4 pmole Na⁺/cm.² sec., 0.25 pmole K⁺/cm.² sec., and 0.5 pmole Cl⁻/cm.² sec.

The protoplasmic Na/K ratio is equal to that in the vacuole but protoplasmic chloride is relatively much lower. Osmotic considerations suggest a layer 4 to 6 μ thick with sodium and potassium concentrations close to those in the vacuole. The fluxes between protoplasm and external solution were of the order of 8 pmoles Na⁺/cm.² sec. and 4 pmoles K⁺/cm.² sec.

We suggest that the protoplasm is separated from the cell wall by an outer protoplasmic membrane at which an outward sodium transport maintains the high K/Na ratio of the cell interior, and from the vacuole by the tonoplast at which an inward chloride transport maintains the high vacuolar chloride. The tonoplast appears to be the site of the principal diffusion resistance of the cell, but the outer protoplasmic membrane probably of the main part of the potential.

INTRODUCTION

There are practically no studies on the ion permeability of plant cells and tissues which are comparable—in the details elucidated—with those made by animal physiologists on nerve, muscle, and erythrocytes. The plant cell with its cell wall, large central vacuole, and thin layer of protoplasm is sufficiently complicated to make such a study difficult but in the plant physiologists' favourite material—roots, storage tissue, etc.—these difficulties are added to by morphological complications. It has thus been possible less often in plant systems to express the results of an experiment in fully quantitative terms—as an ion flux in pmoles (10^{-12} moles) per sq. cm. per second across a well defined morphological boundary separating two phases in which the electrochemical activities of the ion are known. This is the general aim of our work.

Ion uptake by a plant tissue is generally agreed to be a two stage process, a physical non-metabolic entry followed by metabolic transport across a selective barrier, but the nature of these stages and their association with specific morphological features of the tissue cell are very controversial. Some would confine the non-metabolic uptake to the cell walls and extracellular spaces (Levitt, 1957) while others consider that much of the protoplasm is "open" to free diffusion of the ions (Briggs (1957); Briggs and Robertson (1957)). Arisz (1956) suggests that, although the main barrier to ion penetration lies in the tonoplast, entry into the protoplasm is metabolically determined and does involve passage through a diffusion barrier. Electron micrographs (Mercer *et al.* (1955); Farrant *et al.* (1956)) show typical "membranes" at the tonoplast and around the mitochondria but none at the outer boundary of the protoplasm. However, direct observation of the existence of a plasmalemma is hampered by the presence of a cell wall. Transport into the vacuole is generally agreed to be an active process dependent on metabolism (Hoagland and Broyer, 1936) in which the participation of some form of carrier molecule is accepted (Osterhout, 1935). Considerable selectivity towards similar ions has been found, both in early studies and in more recent work (Collander (1936, 1939); Epstein and Hagen (1952); Sutcliffe (1957); MacRobbie and Dainty (1958)). Salt accumulation requires the active movements of either cations or anions but not necessarily of both, for the passive movement of one type of ion can take place down the potential gradient set up by the active transport of the ions of opposite sign. Direct determination of the electrochemical activities of ions on both sides of a permeability barrier across which active transport takes place is needed to settle this question of which ions must be actively transported.

We decided, after an experimental study of ion transport in the marine alga *Rhodomenia palmata*, that quantitative studies on ion movements in plant cells have more chance of success if the complications of the morphology of single plant cells are not supplemented by those due to multicellular organisation. The giant cells of *Nitellopsis obtusa*, a brackish water, ecorticate, characean seemed the most suitable cells available to work with.

Much previous work has been done on members of this group, particularly by Collander, Osterhout, Blinks, Umrath, and others (Collander (1936, 1939); reviews by Osterhout (1955), Blinks (1955), Umrath (1956)). However, these studies have been confined chiefly to chemical measurements of normal concentrations in cell sap and the net uptake from unfamiliar media under a variety of conditions, and electrical measurements of potentials and resistances. Very few kinetic studies have been carried out using tracer techniques. Only these latter will be mentioned here.

Brooks (1940, 1951), whose experiments were few in number and not altogether satisfactory, found that radioactive sodium and potassium entered the protoplasm of *Nitella* more readily than they crossed the tonoplast into the vacuole and that after 6 hours there was no measurable penetration into the

sap. Hoagland and Broyer (1942) measured the rate of penetration of rubidium into *Nitella* and also concluded that the tonoplast was the main barrier to ions. Holm-Jensen, Krogh, and Wartiovaara (1944) measured "permeability" constants, using Na^{24} and K^{42} in *Nitella* and *Nitellopsis*; they concluded that the tonoplast is *more* permeable than the plasmalemma, but a critical analysis of their results indicates the opposite. These kinetic studies are thus fragmentary and are not sufficiently detailed and extensive to give a satisfactory quantitative description of the ion distribution and its kinetics in characean cells. Our work is an attempt to initiate such a study.

Methods

Nitellopsis obtusa was obtained from Snappertuna, Finland, where it occurs in brackish water of chloride concentration 20 to 50 mM. The plants used for the experiments were kept in tanks of aerated brackish water (sea water diluted 15-fold) under cool, not too bright, conditions. They survived such storage for 4 to 6 months before cells died in serious numbers. Rapid protoplasmic streaming and high turgor are sensitive indicators of the health of the cell and only cells showing both these properties were used. Experiments were done on single internodal cells, length 4 to 10 cm. and diameter 500 to 800 μ ; such isolated cells appear to be no less able to survive than the complete uprooted plant. The cells were handled by the cut ends of the neighbouring cells or by tying a thread loosely round the end of the cell where the node prevented its slipping off; this prevented damage to the cell which is very easily destroyed by bending, pinching, etc. in direct handling.

Solutions.—An artificial brackish water similar to the normal external medium of the plant was prepared by combining suitable proportions of N/10 or N/20 NaCl, N/20 KCl, distilled water, and a solution which was equivalent to artificial sea water minus its NaCl and KCl content. The final solution contained the ions of diluted artificial sea water (recipe in Hodgkin and Keynes (1955)) in their correct proportions and could be conveniently labelled by the substitution of radioactive NaCl or KCl in its preparation. Its composition was (mM): Na, 30; K, 0.65; Ca, 0.67; Mg, 3.40; Cl, 35; SO_4 , 1.80; phosphate, 0.09. Cells were isolated from their neighbours and stored in a shallow dish for at least a week in this solution under laboratory conditions of light and temperature before use in experiments. Potassium-enriched solutions were prepared by substituting for part of the NaCl an equivalent amount of KCl, thus keeping $\text{Na} + \text{K}$ constant.

Na^{24} and K^{42} were each obtained from Atomic Energy Research Establishment, Harwell, as the spectroscopically pure carbonates and were titrated with N/20 HCl to pH 7.0. An artificial brackish water was then prepared in the usual way but with the substitution of N/20 K^{42}Cl or N/20 Na^{24}Cl for an equivalent amount of the inactive salt. The activity of these solutions was high, particularly that of Na^{24} , being as much as 150 $\mu\text{C}/\text{ml}$. at the beginning of an experiment. During long experiments with solutions labelled with radioactive sodium the total dose was about 1000 rads. It should therefore be kept in mind that the permeability might not be normal under these conditions, though cells were capable of surviving for several months after larger doses than this, with their protoplasmic streaming apparently unaffected.

Na^{24} was also used in some experiments in which the activity of the solution was

much less (about 10 $\mu\text{c.}/\text{ml.}$) but as the experimental time of uptake was correspondingly longer the total dose was no less in the Na^{22} experiments than in the Na^{24} experiments. The Na^{22} -labelled solution was prepared by adding Na^{22}Cl to 10 ml. inactive artificial brackish water, thereby increasing the sodium concentration by not more than 3 per cent.

Br^{82} -labelled solutions were prepared by the substitution of NaBr^{82} for a part of the NaCl in the uptake solution; pure, radioactive, NH_4Br , obtained from A.E.R.E., was converted to NaBr by the addition of a slight excess of NaOH , boiling to dryness to remove the ammonia, and back-titration of the excess alkali with HCl to pH 7.0. (The bromide content of this solution was taken equal to the total halide although, strictly, chloride is also present to the amount of the excess NaOH originally added.)

Cl^{36} was obtained from Amersham as 1.65 N HCl ; this was titrated to pH 7.0 with N NaOH and the solution diluted to a concentration of 50 mM. The active solution was then prepared from the usual constituents but N/20 NaCl^{36} was used in place of inactive NaCl .

Isotope Experiments.—The procedure was very similar to that used in the experiments on *Rhodymenia* (MacRobbie and Dainty (1958)), except that the same type of washing-out tube was not used. The cells were soaked in radioactive solutions to label the internal ion content; in experiments with short lived isotopes the time of uptake for cells on which a washing-out experiment was done was not usually more than 15 hours, but when only the influx was measured, uptake times of up to 90 hours were possible. With the longer lived isotopes Na^{22} and Cl^{36} , when the decay of radioactivity was no longer the limiting factor, cells were soaked for up to 1 to 2 months in labelled solutions before the start of the washing-out. For the shorter uptakes the cells were under continuous illumination (laboratory fluorescent lighting) and at room temperature, but in long experiments the conditions during the prolonged soaking were less well defined; lighting followed the normal day-night pattern. In general temperature was not controlled except in the Cl^{36} experiment when it was maintained at $20 \pm 1^\circ\text{C}$. Usually the uptake was used only to label the cells; when it was used to calculate an influx the illumination was kept constant.

During washing-out experiments the cells were continuously illuminated and at a room temperature of $17\text{--}20^\circ\text{C}$. In these experiments the cells were in flat, covered perspex dishes in a 0.5 cm. deep layer (10 ml.) of inactive brackish water and the cell was transferred to fresh solution by lifting it from one dish to another either by the cut end of the neighbouring cell or by a loose thread. After the first few minutes of washing-out the frequency of solution changes was gradually decreased from 10 minutes to 2 hours over the first 6 hours of the experiment; later during the slow exchange from the cell sap the time intervals were 2 to 10 hours. A calculation of the rate of removal of labelled ions by diffusion in the bathing solution showed that the ratio of labelled to unlabelled ions in the immediate vicinity of the cell did not rise above 2 per cent during either phase of the exchange. It was therefore considered that the rate of loss of radioactivity, except during the first rapid exchange from the free space of the cell, was slow enough for rapid stirring to be unnecessary.

At the end of the washing-out experiments the cell was measured, the diameter microscopically to about 1 per cent and the length to about 2 per cent from a tracing of the cell on a sheet of paper. The cell was then ashed in 10 ml. of cold N HNO_3 .

or in some experiments a sap sample was removed for counting and chemical estimation and the rest of the cell ashed. (It was checked that this simpler procedure gave the same results as complete ashing in hot concentrated H_2SO_4 and concentrated HNO_3 .)

Liquid counting was used in all but one experiment as described in the previous paper (MacRobbie and Dainty (1958)). The specific activity of the Cl^{36} was rather low for satisfactory liquid counting and in one experiment the chloride samples were precipitated as AgCl on a disc of filter paper and counted by an EHM 2 end-window counter, with an efficiency 5 times higher than M6 liquid counting. Standards were counted under the same conditions for comparison.

Sap Samples.—Sap samples were obtained by rinsing and lightly blotting the cell, cutting off the end with sharp scissors, and gently squeezing out the sap on to a teflon square. The drop of sap was then drawn up into a micropipette and was ready for analysis. Its volume was determined either by using a graduated micropipette and a travelling microscope, or by using a pipette of drawn-down glass with one mark, from which an equal volume of a known standard solution was delivered for comparison. In general 5 to 10 μl . of colourless sap was obtained by this method. This volume of sap was ample for the determination of Na and K by flame photometry and for determination of radioactivity.

Chemical Analysis.—The Na and K in the sap samples were determined using an EEL flame photometer. The 5 to 10 μl . sap sample was added to 5 ml. of distilled water and this solution was analysed by comparison with a standard prepared in a similar way from an artificial sap solution, whose Na and K concentrations were chosen to be similar to those in sap. Various checks indicated that the results were reliable to better than 2 per cent. The Na and K in whole cell were also determined in a number of cases either by "wet-ashing" in N HNO_3 or by dry-ashing in a platinum crucible at 450°C . for 24 hours, followed by flame photometry on suitable solutions.

Chloride was determined to 1 to 2 per cent by electrometric titration by a method similar to that described by Ramsay, Brown, and Croghan (1955).

RESULTS

Chemical Analysis of Sap

Throughout the course of the experiments a large number of sap analyses—of Na, K, and Cl—were done and the results are summarised in Table I, in which the external concentrations are also given for comparison. (Results are quoted in the form: mean \pm standard error of mean (number of determinations).)

Isotope Experiments

The results of the washing-out experiments were treated in the standard manner; the logarithm of the amount of radioactivity left in the cell, or the logarithm of the rate of loss of radioactivity, was plotted against time, a plot in which uniform exchange from a single compartment gives a straight line. In the experiments with *Nitellopsis* the washing-out could be split into three phases and three compartments with distinct efflux rates could be distinguished.

At long times the washing-out curves were linear if plotted in this way and the bulk of the ions of the cell was included in this fraction, but for several hours at the start of the experiment the rate of loss of activity was considerably greater than that of the ions in this slow fraction. Subtraction of the slow fraction from the total activity in the cell gave a second straight line on a semilogarithmic plot, suggesting the presence of a second compartment in the cell with a faster rate of exchange. In addition there was a very rapid loss of radioactivity in the first minute or so which is ascribed to rapid exchange with the free space ions. (The assignment of this very fast fraction to the free space is based on the very high rate of exchange, the fact that the rate of exchange is independent of temperature, and the ratios of the amounts of Na, K, and Cl in this fraction. However, because of certain technical difficulties, we have not yet been able to obtain the absolute amounts of the ions in this free space fraction and hence cannot say what volume of the cell it occupies. Work is being continued on this problem and we hope to publish the results in a later paper.) This present paper

TABLE I
Concentrations of Na, K, and Cl in the Sap and in the External Solution

	Na	K	Cl
Sap concentration range, mM.	26.3 — 77.0	74 — 159	143 — 249
Mean sap concentration, mM.	53.9 \pm 1.6 (63)	113 \pm 2 (69)	206 \pm 3 (45)
External solution, mM.	30	0.65	35

will be concerned with the two "slower" compartments of the cell, which will be discussed separately.

Slow Compartment: Cell Sap

The slow fraction may be fairly definitely associated with the ions of the cell sap; this will be obvious from the results as they are presented but the reasons on which this conclusion is based may be summarised here. These are that the amounts of Na, K, and Cl in the slow fraction of a washing-out experiment correspond with those estimated chemically in the sap, and that the influx into the slow compartment does not differ significantly from the influx into the cell sap determined by direct counting of samples of radioactive sap isolated from a labelled cell.

Potassium Influx.—This was determined, both from the activity in the slow fraction and from the activity of sap isolated from the cell, in the normal external medium (0.65 mM K) and also in external solutions of higher potassium concentration. (Solutions of potassium concentration up to 4 mM were prepared in which the sodium concentration was lower than normal by the amount of the increase in potassium concentration.) The results obtained are summarised in Table II.

Potassium Efflux.—Measurements of the efflux of potassium from this compartment were hampered by the slowness of the ion exchange and the short half-life of the isotope K^{42} (12.4 hours). For this reason the cells on which the efflux was measured were soaked in a radioactive solution of higher potassium concentration than normal so as to produce a reasonably high specific activity of potassium at the beginning of a washing-out experiment.

TABLE II

Potassium Influxes, $\mu\text{moles/cm}^2 \text{ sec.}$ into the Sap from Solutions of Various K Concentrations

K concentrations	0.65 mM	1.3 mM	2.1 mM	4.2 mM
Range, $\mu\text{moles/cm}^2 \text{ sec.}$	0.12 — 0.41	0.7 — 1.95	2.8 — 4.6	4.8 — 8.5
Influx Mean, $\mu\text{moles/cm}^2 \text{ sec.}$	$0.22 \pm 0.02(15)$	$1.5 \pm 0.2(7)$	$3.8 \pm 0.2(9)$	$6.5 \pm 0.4(10)$

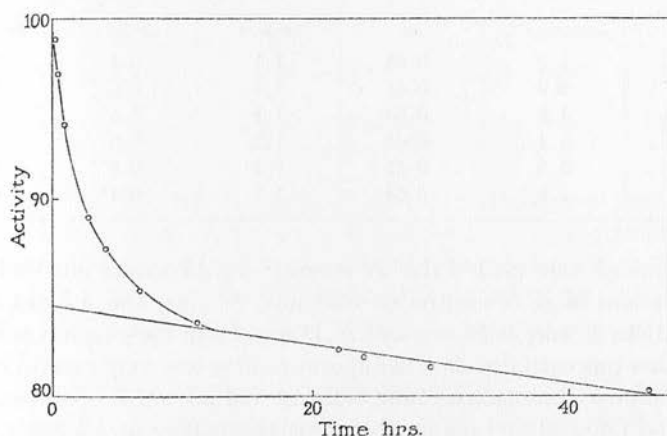


FIG. 1. Exchange of potassium in the cell sap.

Cells were labelled in K^{42} solutions of potassium concentrations 2.1 mM or 4.2 mM and were washed out into normal brackish water of potassium concentration 0.65 mM. One of the washing-out curves is shown in Fig. 1. The mean values of k , the efflux rate constant, for the slow fraction are given in Table III; since k decreased during this time to a steady value, this lower, steady, value is also given. The values of kV/A (V is the cell volume and A the surface area) are also given but, since total K was not determined in each case, the individual effluxes (kV/A times C_i , the internal potassium concentration) cannot be determined.

The mean value of kV/A (calculated from the final, steady, value of k) is $0.89 \pm 0.07 (6) \times 10^{-5}$ cm./hr. which, combined with the mean value of 113 ± 2 mM for the K concentration of the sap, gives an efflux of 0.28 ± 0.03 pmole/cm.²sec. If the mean efflux over 0 to 50 hours is calculated, its value is 0.59 pmole/cm.²sec. Thus the efflux during 50 hours in 0.65 mM K solution after an uptake of about 16 hours in a high K solution is just over twice the normal influx, but by the end of this period the efflux has decreased until it is not significantly different from the normal influx. It seems therefore that, under normal conditions, the fluxes into and out of the sap are equal to each other (and are about 0.25 pmoles K/cm.²sec.) and thus there is flux equilibrium.

Sodium Influx.—The influx of sodium into the sap was determined by means of Na^{24} in the same way as has been described for the potassium influx. Solutions of the same chemical composition as those for which the potassium influx

TABLE III
Exchange of K between Sap and Various External Solutions

K concentration in uptake solution	Influx in uptake solution	K concentration in washing-out solution	Mean k 0-50 hr.	Final k	kV/A
mM	pmoles/cm. ² sec.	mM	10^5 hr.^{-1}	10^5 hr.^{-1}	10^5 cm./hr.
4.2	5.2	0.65	1.1	0.4	0.63
4.2	6.9	0.65	1.1	0.6	1.01
4.2	4.8	0.65	1.1	0.5	0.83
2.1	4.3	0.65	1.1	0.6	0.94
2.1	3.5	0.65	1.1	0.8	1.11
2.1	2.8	0.65	1.1	0.45	0.80

was determined were used. Table IV summarises the values obtained for external solutions of K concentration 0.65 mM, 2.1 mM, and 4.2 mM and Na concentrations 30 mM, 28.55 mM, and 26.45 mM. From these figures an increase in potassium concentration does not appear to have any very marked effect on the sodium influx; the influxes from 0.65 mM and 2.1 mM K solutions are not significantly different and the number of determinations at 4.2 mM is too few for any conclusions to be drawn.

Sodium Efflux.—The efflux of sodium from the sap has been investigated by means of both Na^{24} and Na^{22} . Experiments with Na^{24} are limited by the short half-life of the isotope (15 hours) but on the other hand avoid the difficulties of flame photometry of radioactive samples containing the long lived Na^{22} (half-life 2.6 years). In the Na^{24} experiments, cells were soaked in labelled solution for 13 to 16 hours and the rate of loss of activity from groups of 4 to 5 cells was then followed for 80 hours. Even for 4 to 5 cells the counting rates were very low and some of the counting errors were as high as 10 per cent. After the washing-out the cell was dry-ashed and the total Na determined by flame photometry.

etry. The sap Na was determined by subtraction, from the total Na, of the fast Na; the amount of the latter could be determined from the washing-out curve since it can be assumed to have completely exchanged during the uptake time of 13 to 16 hours. The influxes and effluxes determined from these experiments are necessarily approximate because they are averages for groups of cells; the averaged results of five experiments which gave five influx values and three efflux values were: mean Na influx, 0.39 ± 0.04 (5) pmole/cm.²sec., mean Na efflux, 0.60 ± 0.02 (3) pmole/cm.²sec.

The efflux rate constants were also determined for a number of cells using Na²² but, as no measurements of total sodium were made, this did not give values of the efflux in the individual cells. An approximate value only of the efflux can be found from the rate constant and the mean Na concentration in the sap. As the cells were soaked in Na²² solution for some weeks under rather variable conditions of light and temperature, no influx values could be calculated from the amount of uptake during this time. The efflux rate constants were

TABLE IV
Sodium Influxes into the Sap from Solutions of Various K Concentrations

K concentrations	0.65 mM	2.1 mM	4.2 mM
Range, pmoles/cm. ² sec.	0.09 — 0.50	0.10 — 0.53	0.50 — 0.52
Influx			
Mean, pmoles/cm. ² sec.	0.27 ± 0.03 (20)	0.32 ± 0.06 (7)	0.51 (2)

determined over the period 10 to 120 hours of the washing-out process. Three experiments gave values for $10^5 kV/A$ of 1.7, 2.5, 2.6 (cm./hr.) and combining these with the mean concentration of sodium in the sap (54 mM) gives an average efflux value of about 0.35 pmole/cm.²sec., which, in view of the uncertainties in obtaining this figure, cannot be considered as disagreeing with the Na²⁴ estimate.

Chloride Influx.—Cells were soaked for 40 to 100 hours in Cl³⁶-labelled solution and the influx was determined either by direct counting of sap samples or from the amount left in the cell after washing in inactive solution for 24 hours to remove the activity in the fast fractions. The results of nine experiments (four by sap isolation and five from the slow component) gave Cl influxes ranging from 0.27 to 0.73 with a mean value of 0.45 ± 0.04 (9) pmole/cm.²sec.

Chloride Efflux.—The very low specific activity of the Cl³⁶ available made determination of the efflux of chloride from the sap difficult. In the first experiments the cells were soaked in the Cl³⁶-labelled solution for 6 weeks. Such prolonged soaking was necessary in order to introduce sufficient activity into the cell sap but unfortunately, for a reason which remains unknown, the mortality rate in this solution was high. At the end of the 6 week period some twenty cells

survived and washing-out experiments were done in groups of 4 to 5 cells. All these cells showed protoplasmic streaming and were apparently healthy but the possibility remains that they were not entirely normal. The average values of $10^5 kV/A$ for four groups of cells were 1.1, 0.9, 2.6, 1.6 (cm./hr.) and if these are combined with the average Cl concentration in the sap (206 mM) effluxes of 0.63, 0.51, 1.5, 0.92 (pmole/cm.²sec.) are obtained. Rough influx values were also calculated for the last three groups of cells; they were 0.24, 0.4, 0.4 (pmole/cm.²sec). These figures suggest that there is a net loss of chloride from the cell sap under these conditions although it is small enough to produce only very slow changes in the internal chloride concentration. An approximate calculation shows that, at the end of a 6 week period, the cell chloride had probably decreased by 20 to 30 per cent and therefore the above efflux figures are likely to be an overestimate.

Another set of experiments was done with a Cl³⁶ solution prepared in exactly

TABLE V
Mean Value of λ_0 for Bromide and Chloride

Halide K concentration.....	Bromide 0.65 mM K	Bromide 2.1 mM K	Bromide 4.2 mM K	Chloride 0.65 mM K
Mean $10^5 \lambda_0$, cm./hr.....	$1.6 \pm 0.2(10)$	$3.8 \pm 0.6(3)$	$10.7 \pm 0.7(8)$	$4.6 \pm 0.4(9)$

the same way and in which the cells were soaked for 1500 hours and washing-out experiments were done on 1 to 2 cells. This uptake solution was quite harmless and the cells survived in it quite as well as in the unlabelled medium. Greater counting accuracy was achieved by solid, end-window, counting of AgCl¹³⁶. Five experiments gave values of $10^5 kV/A$ ranging from 0.81 to 0.98 (cm./hr.) and, combining these results with the mean chloride concentration in the sap (206 mM), led to a mean Cl efflux of 0.52 ± 0.01 (5) pmole/cm.²sec. No influx determinations were made on these cells.

Bromide Influx.—Since the specific activity of Cl³⁶ was so low the movements of halide into the cell were also investigated by means of Br⁸². (Time was allowed for the short lived Br⁸⁰ (18 minutes and 4.4 hours) to decay before any counting was attempted.)

The influx of bromide into the sap was rather variable and the reason for the wide scatter of the results is not known. Since not all the chloride in the normal solution was replaced by active bromide and the external bromide concentration varied in different experiments, the most significant quantity obtained from these influx measurements is the "permeability" constant, λ_0 (cm./hr.), equal to the influx divided by the external bromide concentration. This allows the behaviour of bromide to be compared with that of chloride. Some of these experiments were done with solutions of different K concentration; the total

halide concentration was 35 mM in each case. The results are summarised in Table V in which λ_0 for Cl is given for comparison. From these results it appears that bromide is not a satisfactory tracer for chloride and that it enters the sap less readily than chloride.

Rubidium Fluxes.—The rates of entry and loss of rubidium were determined by means of Rb^{86} . Cells were soaked for 1150 hours in an artificial brackish water containing 1 mM Rb^{86}Cl in addition to the normal constituents. Washing-out experiments were then done in the usual way. The experimental results are given in Table VI, together with some comparative figures for potassium. The influx of rubidium is very low compared with that of potassium and the rate of washing-out of Rb is lower than that of K although the difference is less than in the case of the influx.

TABLE VI

Movements of Rb to and from the Cell Sap, Together with Comparable Figures For K

	Influx	$10^3\lambda_0$	10^3k	$10^3kV/A$
	$\mu\text{moles/cm}^2 \text{ sec.}$	cm./hr.	hr.^{-1}	cm./hr.
Rb 1 mM {	0.031	11.2	0.25	0.45
	0.028	10.1	0.17	0.25
	0.049	17.6	0.42	0.66
K, 0.65 mM	0.22	122	0.5	0.89
K, 1.3 mM	1.5	415	2.0	3.6

λ_0 , the "permeability" constant in cm./hr. for inward movement, equal to the influx divided by the external concentration. k , the rate constant for washing-out, obtained from the slope of the semilogarithmic plot of the activity in the cell against time. kV/A : the permeability constant in cm./hr. for outward movement.

Protoplasmic "Non-Free" Space

As pointed out earlier a *Nitellopsis* washing-out curve is the sum of two exponentials and a diffusion curve or, approximately, the sum of three straight lines when the curve is plotted logarithmically. After subtracting the "slowest" exponential we obtain, in general, a curve similar to that of Fig. 2, which is clearly the sum of an exponential and a very steep curve. We consider that the slowest exponential corresponds to efflux from the cell sap, as indicated above, and that the ions represented by the remainder of the curve are outside the cell sap. Those in the very steep part we identify with the ions of the "free space." We consider the ions in the middle fraction—half-time of the order of an hour—to be in a compartment we shall call "protoplasmic non-free space." One difficulty in discussing this compartment is that its volume is not known. For presentation of the results we shall use the amount of ion divided by the cell volume but this is an arbitrary procedural choice and we shall discuss later what the volume of this compartment is likely to be.

From washing-out experiments with Na^{24} and Na^{22} , the amount of Na, expressed as the amount per liter cell volume, and the half-time for exchange found from the slope of the semilogarithmic plot of this fraction, were found to be 0.8 ± 0.1 (18) mM and 24 ± 2 (18) minutes. The results, expressed in this way, were very variable; the Na concentrations ranged from 0.25 to 2.2 mM and the half-times from 11 to 67 minutes.

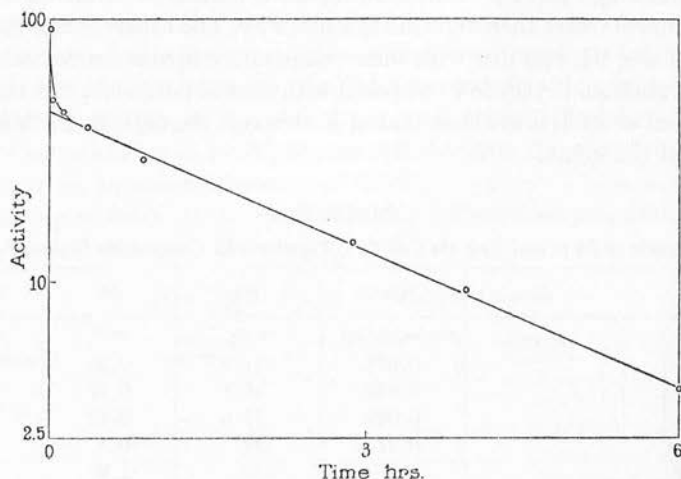


FIG. 2. Exchange of potassium in protoplasmic non-free space.

TABLE VII
Potassium in Protoplasmic Non-Free Space

Uptake solution (mM K)	Amount of K/liter cell volume mM	$T_{1/2}$ at 20°C. (hrs.) washing-out into 0.65 mM K	$T_{1/2}$ at 2°C. (hrs.) washing-out into 0.65 mM K
0.65	1.4 ± 0.2 (8)	1.65 ± 0.25 (3)	11.35 ± 0.05 (3)
2.1	4.6 ± 0.2 (3)	1.64 (2)	
4.2	5.7 ± 0.7 (7)	1.89 ± 0.10 (3)	

Similar experiments were done with K^{42} . However, in this case the uptake solutions, for labelling the cells, were of various K concentrations; washing-out was done into the normal 0.65 mM K solution. The results are summarised in Table VII.

The relative amounts of Na and K in this compartment are of considerable interest. The above experiments indicate a ratio of K/Na of 1.8, but this ratio is more readily determined by an experiment in which Na^{22} and K^{42} are used simultaneously, when the wide variations among the cells do not obscure the results. Cells were soaked in a solution labelled with Na^{22} , K^{42} , and Br^{82} for 12 h

15 hours and then washing-out experiments were carried out from which the amounts of all three ions in this fraction of the individual cell were determined. The amounts of Na and K and their ratio for three cells are given in Table VIII. It is clear that there is about twice as much potassium as sodium in the protoplasmic non-free space. (The external K/Na ratio is 0.022.)

The amount of rubidium taken up in this compartment from an external solution containing 1 mM Rb⁸⁶ was also determined from the washing-out curves in inactive 1 mM Rb solution after an uptake of 1150 hours. Four experiments gave mean values of 0.21 ± 0.07 (4) mM for the amount of Rb per liter cell volume, and 1.37 ± 0.22 (4) hour for the half-time.

Very few estimates of the amount of chloride or bromide in this fraction have been made. Five determinations of the amount of chloride were made from the activity, in excess of the loss from the sap, lost between 2 minutes and 7.5 hours of a washing-out experiment. The mean result was that the amount of chloride per liter cell volume was 0.32 ± 0.07 (5) mM. The specific activity was too low

TABLE VIII

Amounts of Na and K and Their Ratio in Protoplasmic Non-Free Space, Determined Simultaneously on Same Cell

Amount of Na/liter cell volume	Amount of K/liter cell volume	K/Na
mM	mM	
0.68	1.8	2.6
0.60	0.92	1.5
0.25	0.47	1.9

for convenient determination of the rate of exchange of this fraction by means of Cl³⁶. A number of experiments with Br⁸² indicated that the half-time for exchange of "halide" was between 20 and 40 minutes.

DISCUSSION

The most straightforward interpretation of the main results of our experiments is that external ions are first exchanged with free space ions which are distributed according to a Donnan equilibrium; then exchange takes place between free space ions and protoplasmic non-free space ions and finally the protoplasmic non-free space ions exchange with ions in the vacuole across the tonoplast. The latter two exchange processes are not necessarily "passive," physical, processes; our results on fluxes, amounts, and concentrations enable us to decide which ions are probably passively exchanged and which involve some "active" transport.

The figures for the ion concentrations in the sap, together with the observation that the tonoplast is permeable to ions in some form, imply the existence in the cell of a mechanism of active transport for some of the ions, although the

sites of this transport cannot be determined from this information alone. If an ion is moving *passively* from an external solution in which its concentration is C_o to an internal solution in which its concentration is C_i , then the net flux (inwards) of the ion is given by:

$$M = P \{C_o - C_i \exp (zFE/RT)\} \quad (1)$$

in which M is the flux in moles/cm.²sec.; z is the charge, in units of the electron charge, on the ion; F is the faraday; R the gas constant, and T the absolute temperature; E is the electrical potential difference between the inside and outside solutions; P is a permeability factor depending on the properties of the membrane separating the two solutions (see chapter 11 in Johnson, Eyring, and Polissar (1954)). Equation (1) is the equivalent, for ions, of Fick's diffusion equation. We are making the usual approximation of replacing activities by concentrations; this should lead to no serious error when dealing with two aqueous solutions such as the external, brackish solution and the sap.

TABLE IX
Ion Concentration Potentials (E) in the Sap

Ion	C_o/C_i	$E = \frac{58}{z} \log \frac{C_o}{C_i}$ mv.
Na ⁺	0.556	-15
K ⁺	0.00575	-130
Cl ⁻	0.170	+45

Our results indicate, as would be expected, that when a *Nitellopsis* cell is bathed by its normal external medium the influx of any ion into the sap equals the efflux; *i.e.*, the net flux is zero. Thus any ion moving passively between the external solution and the sap must have its concentrations governed by the equation:

$$M = P \{C_o - C_i \exp (zFE/RT)\} = 0$$

$$\text{i.e. } E = \frac{RT}{zF} \ln \frac{C_o}{C_i} = \frac{58}{z} \log \frac{C_o}{C_i} \text{ mv.} \quad (2)$$

In Table IX we give the values E (the ion concentration potential) should have if the ions concerned move passively under the action of the purely physical forces of chemical and electrical potential gradients. Since the vacuolar potential must have a unique value, it is clear that at least two of the three ions must be actively transported between external solution and sap.

Recently in this department Dr. Williams and Dr. Johnston measured the potential difference between the vacuole of *Nitellopsis* and the normal brackish external medium. Their results range from 120 to 200 mv. with the vacuole negative. It would thus appear that whereas it is possible that the potassium ion in the vacuole is in electrochemical equilibrium with the external potassium,

both sodium and chloride are very far from their equilibrium distribution. Thus sodium and chloride must be undergoing active transport and it is clear from an examination of Table IX that sodium is actively transported outwards and chloride actively transported inwards.

The probable sites of these transports in the system of compartments found in the tracer experiments may be deduced from the relative amounts of the three ions in each compartment and the probable relations of the tracer compartments to cell morphology may then be considered. The reasons for the association of the slow compartment with the cell sap have already been given. The volume of the second compartment, the protoplasmic non-free space, is uncertain but some of its properties can be discussed without specifying its volume. The K/Na ratio in this compartment is about 2 and does not differ significantly from the K/Na ratio in the vacuole. This suggests that the sodium-potassium selectivity is a property of the protoplasmic non-free space. We suggest that this selectivity is a property of a membrane separating this space from the free space and that it is due to an outwardly directed sodium pump of the kind postulated by Hodgkin (1951) to explain the same kind of selectivity in nerve and muscle. The ratio $(\text{Na} + \text{K})/\text{Cl}$ is about 7 and may indeed be much higher as our figure for protoplasmic chloride is likely to include contamination with free space chloride; this figure differs markedly from the vacuolar ratio of 0.8. This, together with our later estimates of ion concentrations in the non-free space, suggests that the inwardly directed chloride pump is located at the tonoplast, between the protoplasmic non-free space and the vacuole.

We may speculate about the size of the protoplasmic compartment from osmotic considerations, since it is presumably in osmotic equilibrium with the vacuole. The only "facts" available are the total amount of monovalent cation—2.2 m. mole/liter total cell volume—and the osmolarity—approximately 400 mM. Reasonable guesses have to be made about whether the balancing anions are fixed or mobile and about the activity coefficients of the monovalent and divalent cations. Depending on these assumptions the values for $(\text{Na} + \text{K})$ concentration would range from 120 to 200 mM if the ion activity coefficient were unity and would be correspondingly greater for lower activity coefficients. These figures lead to estimates of protoplasmic non-free space volume ranging from $1\frac{1}{2}$ to 1 per cent of the cell volume (or less if the ion activity coefficients are low). For a typical cell of length 5 cm. and diameter $600\ \mu$ the protoplasmic non-free space, if it were spread in a single layer near the cell wall, would be 2 to $3\ \mu$ thick (or less if there was appreciable cation binding). This figure for the thickness must be increased if allowance is made for the osmotically inactive solids in the protoplasm; an increase of up to a factor 2 is possible, leading to an estimated thickness of 4 to $6\ \mu$.

Of the several possibilities for the location of the protoplasmic non-free space the most likely seem to be: (a) the whole of the protoplasm; (b) a 4 to $6\ \mu$

thick layer bordering the tonoplast, the flowing protoplasm perhaps; (c) some or all the chloroplasts, mitochondria, etc., which would then be embedded in the so called protoplasmic free space of the cell.

All these possibilities have their virtues: (a) would be in conformity with the current picture of external membrane-limited animal cells, although the estimated protoplasm thickness is only just within the range of previous estimates in Characeae, from 5 to 15 μ (Collander (1930), Holm-Jensen, Krogh, and Wartiovaara (1944), Peebles (1956)); (b) is compatible with estimates of the thickness of the flowing protoplasm and might fit in with Arisz's symplasm theory; (c) has been discussed by Robertson (1956), who gives reasons for considering the mitochondria as the sites of ion selectivity and accumulation. We prefer possibility (a) (or (b)), partly to conform with the accepted picture of animal cells; also if the protoplasmic non-free space is confined to the mitochondria a substantial proportion of the cations would have to be bound (this is in agreement with Robertson's ideas); in addition our protoplasmic non-free space exchange rates are 50 to 100 times slower than isolated beet mitochondria exchange rates. Finally it is difficult to reconcile the amounts and relative proportions of the various ions in the two phases with the concept of mitochondria as ion carriers across the tonoplast. On the basis of (a), a provisional scheme for the normal state is given in Fig. 3 showing the ion concentrations and fluxes in the two compartments. The somewhat speculative concentrations in the protoplasm and fluxes into the protoplasm are given in parentheses. The tonoplast fluxes and vacuolar concentrations are not so uncertain. The scheme assumes the ion pump hypothesis.

On this picture the protoplasm is separated from the cell wall, a Donnan system, the "free space", by an outer protoplasmic membrane which seems to be similar to a typical animal cell membrane, and from the vacuole by the tonoplast, a typical plant cell membrane. At the outer membrane an outward sodium pump, perhaps coupled to an inward potassium transport as has been suggested in animal cells (Hodgkin and Keynes, 1955), maintains the high K/Na ratio of the protoplasm. The fluxes (influx and efflux) of Na and K across this membrane, based on this scheme, are about 8 pmoles Na/cm.²sec. and 4 pmoles K/cm.²sec. Assuming that the Na and K *influxes* are both passive we can deduce a K/Na permeability ratio of about 23. These fluxes are rather lower than those in squid nerve but similar to those in muscle (Hodgkin, 1951). The permeability ratio is also similar to those found in animal cells (Conway (1957); Hodgkin (1951)), and the low protoplasmic chloride implies the existence of indiffusible anions in the protoplasm as in animal cells.

Up to the tonoplast the system seems very similar to the typical animal cell but the tonoplast is a membrane with very different properties. There is little discrimination between sodium and potassium at this membrane and it is therefore likely that the cation fluxes across the tonoplast are entirely passive. From the fluxes and concentrations, the tonoplast is slightly more permeable to

sodium than to potassium, in marked contrast to the plasmalemma and animal cell membranes which are much more permeable to potassium than to sodium. Also the tonoplast is much tighter to ions than the outer protoplasmic membrane and than animal cell membranes, for the fluxes across the tonoplast are 20 to 100 times lower. The tonoplast must therefore be the site of the principal diffusion resistance of the plant cell. The tonoplast fluxes lead to a calculated value of the electrical resistance between vacuole and external medium of



		PLASMALEMM A (?)	TONOPLAST		
MEDIUM		FREE SPACE CELL WALL [+ SOME CYTOPLASM ?]	PROTOPLASMIC NON-FREE SPACE	SAP	
<u>Na</u>	30		(54)	54	CONCENTRATIONS MM.
<u>K</u>	0.65		(113)	113	
<u>Cl</u>	35		(25)	206	
<u>Na</u>			(8)	0.4	FLUXES P MOLES / CM. ² SEC.
<u>K</u>			(4)	0.25	
<u>Cl</u>				0.5	

FIG. 3. Provisional scheme for the normal state of the *Nitellopsis* cell showing ion concentrations and fluxes in the two compartments. Protoplasmic figures, shown in parentheses, are uncertain as the volume of this compartment is not accurately known.

about 250,000 ohm cm.², in agreement with the estimate of Blinks (1930) but not with those of Bennett and Rideal (1954) and Walker (1957).

Some predictions of the partition of the total potential of the cell between the two membranes may be made for the proposed system but so far we have not been able to measure the potential across either membrane separately. Since Na and K appear to be passively distributed across the tonoplast in approximately equal concentrations, there should be only a small potential difference (see equation (2)), and thus the main potential drop would be expected across the external protoplasmic membrane. Walker's (1955) measurements on *Nitella* support this deduction and, if so, it is interesting that the main potential drop and the main resistance seem to be associated with different membranes.

The energy requirements for the proposed system of pumps may be calculated (see Keynes and Maisel (1954)). The minimum work associated with an active sodium efflux of 8 pmoles/cm.²sec. at the outer membrane is about 80×10^{-3} cal./gm. protoplasm per hour and with an active chloride influx at the tonoplast of 0.5 pmole/cm.²sec. is about 9×10^{-3} cal./gm. protoplasm per hour. A total energy requirement of 9×10^{-2} cal./gm. protoplasm per hour should be well within the metabolic capacities of the cell.

The effects of changing the external potassium concentration are very striking. A detailed analysis of the results, which will be published later after further experiments, indicates that they can all be best explained by a general increase of the tonoplast permeability to *all* ions.

The difference in behaviour between potassium and rubidium is rather surprising since the ions are much the same size and are usually considered as biological near equivalents. The results show that the difference is due to the much lower permeability of rubidium, as compared with potassium, at the outer protoplasmic membrane. At the tonoplast it seems to have the same permeability as sodium and potassium. This might suggest that a considerable part of the inward potassium movement at the outer membrane was active and that a linked sodium-potassium pump of the type proposed for squid nerve was operating here.

We are grateful to Dr. E. J. Williams for general help, to Dr. V. Wartiovaara for supplies of *Nitellopsis*, and to Dr. R. N. Robertson and Dr. A. B. Hope for helpful discussion.

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THE WATER PERMEABILITY OF CELLS OF CHARA AUSTRALIS¹

by

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Summary

Measurements have been made of the osmotic permeability (P_{os}) of internodal cells of Chara aust. using the method of transcellular $\underline{P_{os}}$ osmosis. The mean value was $5.6 \mu^3/\mu^2 \times \text{min.} \times \text{atmos.}$, which is close to that obtained by Kamiya and Tazawa (1956) with cells of Nitella. A small difference between rates of water movement into and out of the cell is ascribed to an asymmetry of the driving forces in the system and not to a real difference in permeability.

Attempts to measure the diffusional permeability (P_d) of similar cells by studying the isotopic exchange of D_2O for the cell $\underline{H_2O}$ were not successful for reasons discussed.

The basis for comparison of P_{os} with P_d is discussed with particular reference to concepts of "filtration" and "bulk flow" of water through pores in the cell membrane.

I. INTRODUCTION

The water permeability of plant cells has, until recently, been studied by methods involving plasmolysis and deplasmolysis. The experiments of Osterhout (1949 (a), (b)) and the important paper of Kamiya and Tazawa (1956) have introduced a new method - transcellular osmosis - which is applicable to large cylindrical cells and which does not involve plasmolysis. In this

¹ J.Aust.Biol.Sci. 1959 - in the press.

paper we describe the application of this method to the study of the "osmotic" water permeability P_{os} of the large, single internodal cells of Chara australis v. nobilis; we have also tried to measure with the same cells the "diffusional" water permeability, P_d , by studying isotopic exchange of the cell water.

Both these permeabilities have been studied separately on Characean cells, the osmotic permeability of Tolypellopsis (now named Nitellopsis) by Palva (1939) and of Nitella by Kamiya and Tazawa (1956), and the diffusional permeability of Tolypellopsis by Wartiovaara (1944). A comparison of the P_{os} and P_d values of several different animal cells (amoeba, various fish and amphibian eggs: Prescott and Zeuthen, 1953; red blood cells: Paganelli & Solomon, 1957) has been used to make deductions about the size and number of pores in the cell membrane.

In the present experiments we have obtained a value for the osmotic permeability of internodal cells of Chara australis, but attempts to measure diffusional permeability failed for reasons set out in the discussion. However, the merits of a comparison between P_{os} and P_d are discussed because it seems to us that a number of misconceptions about the meanings of osmotic and diffusional permeability are current.

II. THEORY OF TRANSCELLULAR OSMOSIS

In the theories of transcellular osmosis which have already been published - a simple version by Osterhout (1949 (a), (b)) and a detailed theory by Kamiya and Tazawa (1956) - the role of the changes in turgor pressure has not been considered and hence, in certain respects, these theories are inadequate. In the following paragraphs we give a simple account of transcellular osmosis which we believe is a more correct description of the phenomenon than previous accounts.

Fig. 1

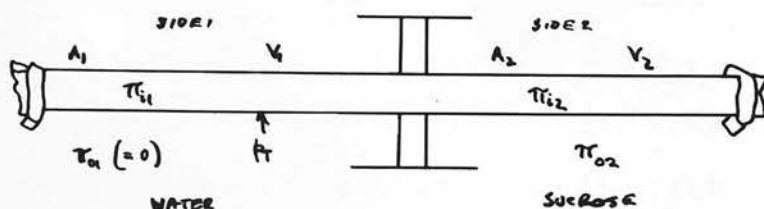


Fig. 1: The situation during transcellular osmosis which is discussed in section II:

$\pi_{i1,2}$ are internal osmotic pressures due to vacuolar solutes on side 1 or 2. $\pi_{o1,2}$ are external osmotic pressures (zero on side 1 which contains water). P_T is the turgor pressure, constant along the length of the cell.

Certain assumptions have, however, been made. These are that the cell is perfectly semi-permeable, i.e., the permeability to water is infinitely greater than that to solutes such as sucrose and the ions in the cell vacuole. This in turn assumes no active mechanisms involving water transport and that the ion transport mechanism(s) contribute negligible fluxes compared with the fluxes of water. These assumptions are probably well founded (Mercer, 1955; Dainty and MacRobbie, 1958). The movement of water along the cell wall is assumed to be negligible (see Kamiya and Tazawa, 1956). The experimental arrangement is shown diagrammatically in Fig. 1, where the symbols used are also given.

When distilled water is bathing both ends of the cell, there will be no net suction tension and the cell will have a turgor pressure, p_T , equal to Π , the initial osmotic pressure of the sap solution. At $t = 0$ the water at end 2 of the cell is replaced by a sucrose solution of osmotic pressure Π_{02} atmospheres. (The time necessary to change the solutions and the time for the sucrose to diffuse to the surface of the cell are neglected.) Then at $t = 0$ the suction tension at end 1 is still zero, but at end 2 it is equal to $\Pi_{02} - (\Pi - p_T) = \Pi_{02}$. Thus water starts to move out from the cell at end 2 at an initial rate given by $P_{os} \cdot A_2 \cdot \Pi_{02}^*$, where P_{os} is the osmotic permeability of the cell 'membrane' in cm/sec.atmos. and A_2 the cell area at end 2. There is no initial movement of water into end 1 of the cell.

The effect of a movement of Δv cc of water out of end 2 is to decrease the volume of water in the cell and hence to reduce the turgor pressure of the cell by Δp_T . The volume of water in the compartment around end 1 would apparently decrease because of cell shrinkage and indicate an apparent movement of water through the cell equal to $\Delta v \cdot V_1/V$ where V_1 is cell volume in end 1 and V total

* Our results indicate some difference between the rates of water movement into and out of the cell, but we shall assume the same permeability coefficient P_{os} for both endosmosis and exosmosis for reasons given in the discussion.

cell volume. The decrease Δp_T in turgor pressure would start a real flow of water into end 1 given by $P_{os}.A_1. (\Pi - \overline{p_T} - \Delta p_T)$. This would be small compared with the flow of end 2 if the cell were very extensible, i.e., if $\Delta p_T / \Delta v$ were small; in this case the apparent initial rate of flow of water through the cell would be given by $P_{os}.A_2. \Pi_{O2}. V_1/V$ which equals $P_{os}.A_1A_2. \Pi_{O2}/A$. The cells of Chara australis are not very extensible; an outflow of a few tenths of a μl produces a change in turgor pressure of 7 or 8 atmospheres. Thus a net outflow of a fraction of a μl will produce such a large drop in turgor pressure that an appreciable inflow of water almost immediately occurs into end 1 and the steady state in which

$$P_{os}.A_1 (\Pi_{i1} - p_T) = P_{os}.A_2. (\Pi_{O2} - \overline{\Pi_{i2}} - p_T) \quad (1)$$

is reached after a net outflow of a few tenths of a μl .

Π_{i1}, Π_{i2} are the osmotic pressures in the cell on sides 1 and 2 respectively.

It is clear from equation (1) that when the steady state has been reached, the turgor pressure of the cell has decreased to

$$\begin{aligned} p_T &= \frac{A_1 \Pi_{i1} + A_2 \Pi_{i2}}{A_1 + A_2} - \frac{A_2}{A_1 + A_2} \cdot \Pi_{O2} \\ &= \Pi - \frac{A_2}{A} \cdot \Pi_{O2} \end{aligned} \quad (2)$$

We can write for the steady state rate of flow, dv/dt , the following equations:

$$\frac{dv}{dt} = P_{os}.A_1. (\Pi_{i1} - p_T) \quad (3)$$

$$\frac{dv}{dt} = P_{os}.A_2. (\Pi_{O2} - \overline{\Pi_{i2}} - p_T) \quad (4)$$

and these two equations can be combined to one equation:

$$\frac{dv}{dt} = K (\pi_{i1} - \pi_{i2} + \pi_{o2}) \quad (5)$$

$$\text{where } K = P_{os} \cdot \frac{A_1 A_2}{A_1 + A_2} \quad \text{or} \quad P_{os} / \left(\frac{1}{A_1} + \frac{1}{A_2} \right) \quad (6)$$

From equation (5) Kamiya and Tazawa (1956) have developed a detailed theory of transcellular osmosis. Qualitatively, water flows into end 1 and then moves along to end 2, inside the cell, carrying with it a proportion of the internal solutes; pure water leaves at end 2. Thus the water flow results in an increasing polarisation of the sap solution; it becomes more concentrated at the sucrose end (2) and more dilute at the water end (1). This polarisation has been directly determined by Kamiya and Kuroda (1956). As can be seen from equation (5) polarisation of the internal solutes decreases the rate of flow of water which would cease when polarisation had produced zero suction tensions at the two ends of the cell. This final state is never quite reached because the polarisation is opposed by solute diffusion and by stirring of the sap by the protoplasmic streaming.

Kamiya and Tazawa (1956) give a quantitative theory of the events described above which fits their results reasonably well. However, the theory contains certain simplifying assumptions and does not take into account the complicated geometry of the system. We have, therefore, thought it preferable to use only the initial rates of water movement as a measure of permeability. This initial rate is given by

$$\frac{dv}{dt} = K \cdot \pi_{o2} \quad (7)$$

$$\text{hence } P_{os} = K \left(\frac{1}{A_1} + \frac{1}{A_2} \right) = \frac{dv/dt}{\pi_{o2}} \left(\frac{1}{A_1} + \frac{1}{A_2} \right) \quad (8)$$

Equation (7) contains no doubtful theoretical assumptions, but it is necessary to ensure that there is no appreciable polarisation of the internal

Fig 2

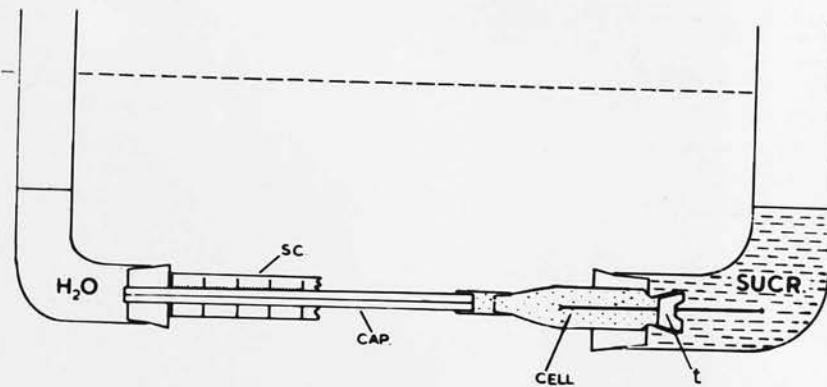


Fig. 2: The form of apparatus used to measure the rate of water movement during transcellular osmosis. It is very similar to that of Osterhout (1949).

sc. = millimeter scale. cap. = capilliary.

t. = split perspex taper with a central 1.6 mm dia.

hole in which the cells are sealed with vaseline.

Distilled water is shown dotted, sucrose striped.

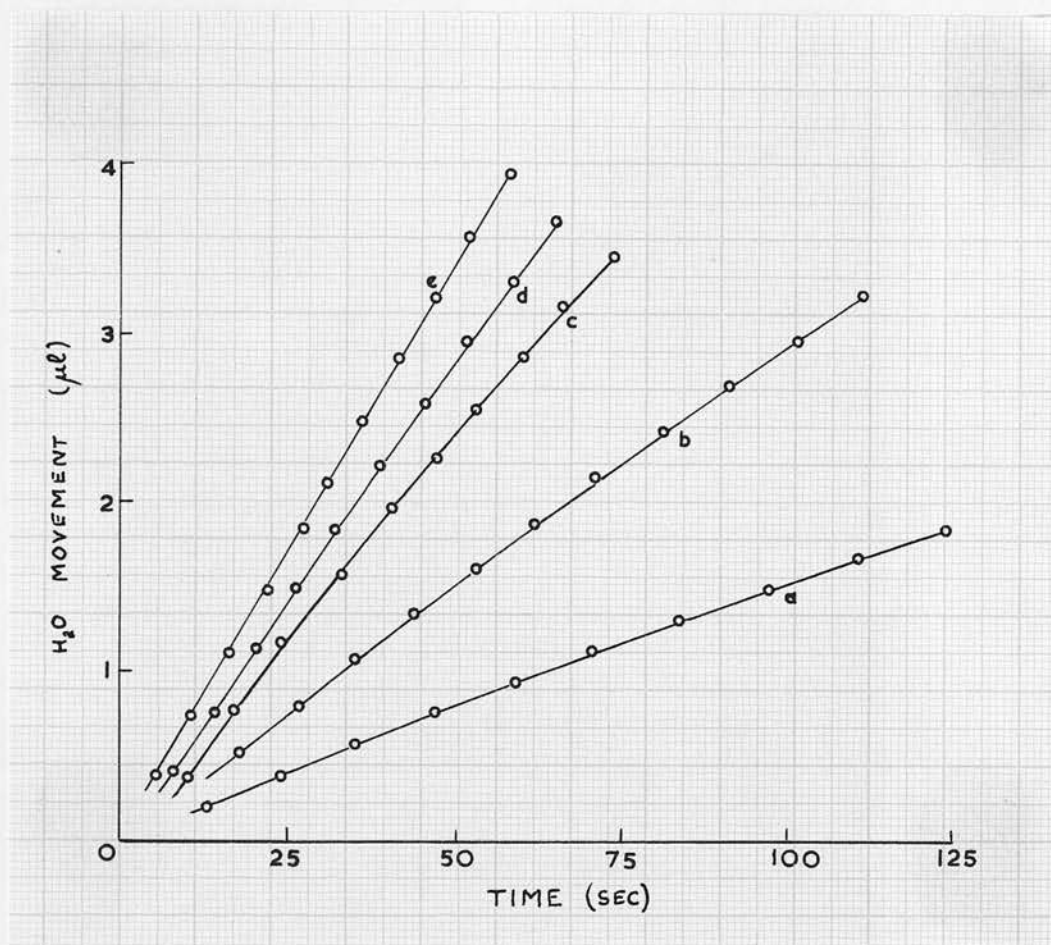


Fig. 3: The volume of water transferred during transcellular osmosis in μl plotted against time in seconds. In curves a - e, the osmotic gradients between sides 1 and 2 were due to 0.1, 0.2, 0.3, 0.4, and 0.5M sucrose respectively being placed on side 2. The areas on sides 1 and 2 were equal.

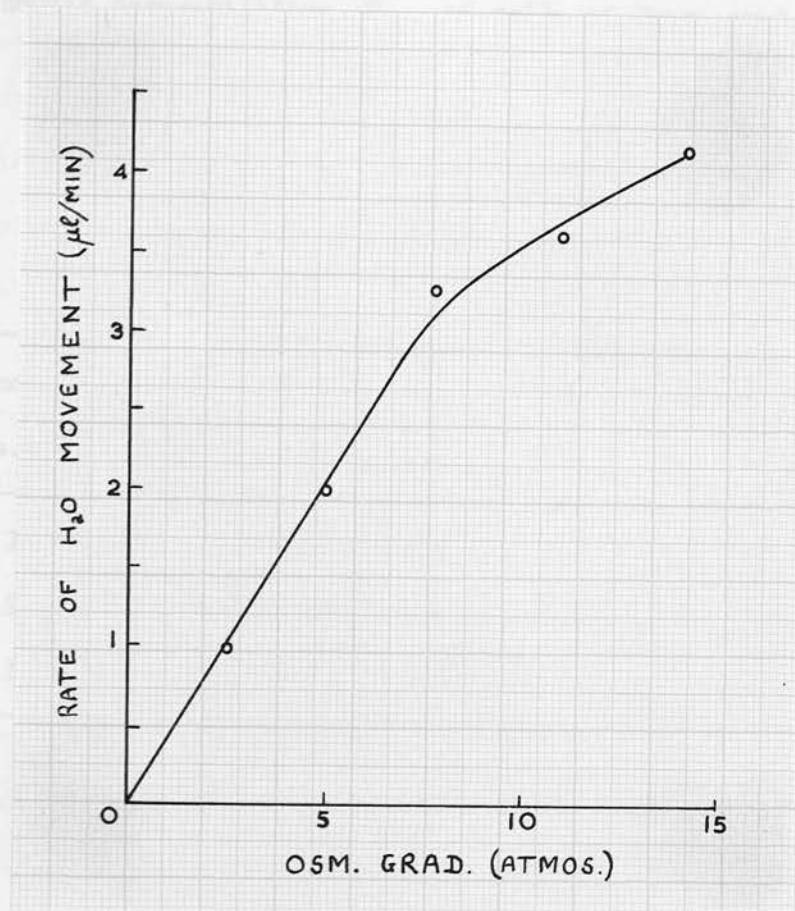


Fig. 4: The relation between rate of water transfer (in $\mu\text{l}/\text{min}$) and the osmotic gradient (in atmos.).
Data from cell 3 in table 1.

TABLE 1

Osmotic permeabilities, P_{os} , of cells of *Chara aust.*

in $\mu^3/\mu^2 \times \text{min.} \times \text{atmos.}$

Sucrose concn. (M/L)	0.05	0.1	0.2	0.3	0.35	0.4	0.5
O.P. (atmos.)	1.25	2.5	5.0	7.8	9.3	11.0	14.2
P_{os} : Cell 1	-	4.7	4.2	-	3.6	-	3.7
2	5.2	6.1	5.6	5.2	-	4.5	4.4
3	-	6.2	6.3	6.6	-	5.2	4.6

IV. RESULTS

(a) Real cell areas in sucrose and water

Figs. 3 and 4 show results from a typical cell. In Table 1 the results from three different cells for various osmotic pressure gradients are collected. P_{os} was calculated from equation (5). During the initial few seconds, the rate of flow was low because of the time required, on changing from water to sucrose solution,

solutes during the period of measurement.

III. EXPERIMENTAL METHODS

The osmotic permeability was calculated from the rate of water movement through a measured area of cell surface under a known osmotic gradient, using equation (8), the rate being measured during transcellular osmosis. The apparatus used for most of the experiments was similar to that of Osterhout (1949, (a), (b)) and is illustrated in Fig. 2. The apparatus and flasks of solution were immersed in a water bath at $25 \pm 0.01^\circ\text{C}$. The condition of the cells, as judged by the protoplasmic streaming, could be checked at any time with the aid of a binocular microscope. The cells were not damaged by overall osmotic gradients of up to 14 atmospheres; at this value occasional 'splitting' of the ordered chloroplast layers was observed. Sucrose solutions were used to produce the osmotic pressure gradients. One centimetre travel of the air bubble in the capillary corresponded to a transfer of $0.912 \mu\text{l}$ of water.

The rate of exchange of D_2O with H_2O was measured from the change in reduced weight with time after the cell was transferred from a D_2O - H_2O mixture, in which it had equilibrated, to a large volume of water. This method is analogous to that of Løvtrup and Pígon (1951). Similar experiments for comparison were done with agar cylinders of the same dimensions as the cells.

IV. RESULTS

(a) Equal cell areas in sucrose and water

Figs. 3 and 4 show results from a typical cell. In Table 1 the results from three different cells for various osmotic pressure gradients are collected. P_{os} was calculated from equation (8). During the initial few seconds, the rate of flow was low because of the time required, on changing from water to sucrose solution,

for diffusion of the sucrose solution to the surface of the cell. Therefore, in calculating the initial rate of flow from the initial slope of the curve of volume transferred vs. time, the initial point and sometimes the second point were ignored.

The mean value of P_{os} from these experiments is $5.6 \mu^3/\mu^2 \cdot \text{min. atmos.}$ If osmotic pressure

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(b) Unequal or

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ratio of the areas was 5:1. This and six other measurements led to an apparent

ratio of the osmotic permeabilities in the inward and outward directions of 1.4 ± 0.1 .

if P_{os}^1 is the permeability for inward water movement and P_{os}^2 is the permeability

for outward water movement it can easily be seen from appropriate modifications

of equation

Fig. 5: The volume of water transferred (in μl) plotted against time (in secs.) when the areas of the cell in

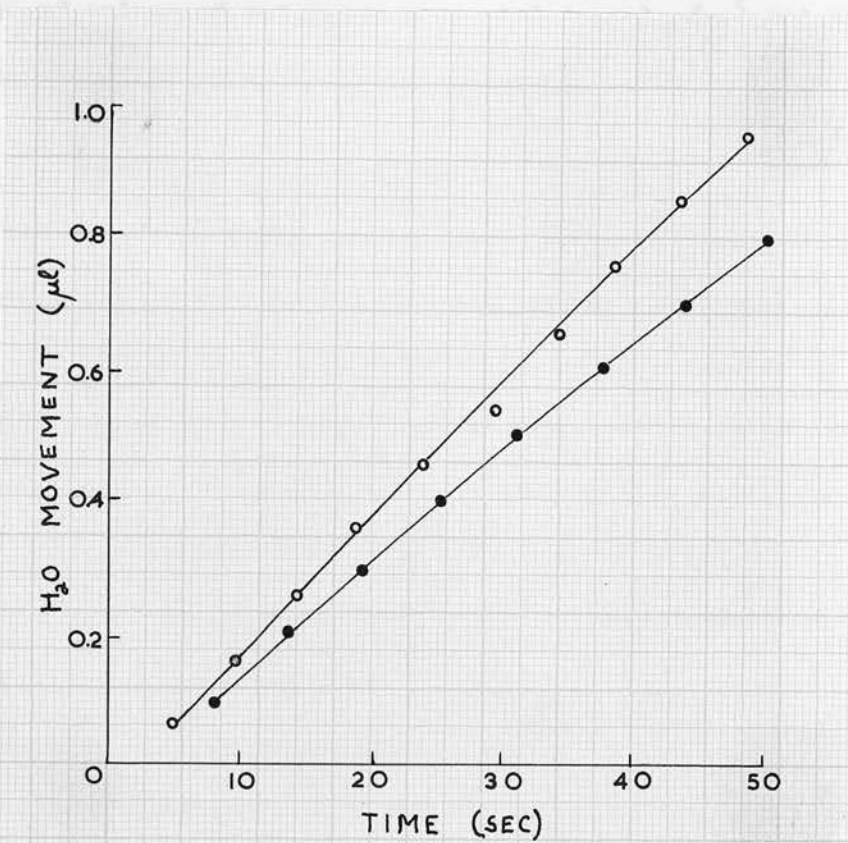
compartments 1 and 2 were unequal: Closed circles

0.2M Sucrose at the shorter end. Open circles 0.2M Sucrose

at the longer end. The ratio of the areas was 5:1.

where A_1/A_2 is the ratio of the areas

rate of water flow



for diffusion of the sucrose solution to the surface of the cell. Therefore, in calculating the initial rate of flow from the initial slope of the curve of volume transferred vs. time, the initial point and sometimes the second point were ignored.

The mean value of P_{OS} from these experiments is $5.6 \mu^3/\mu^2 \cdot \text{min.atmos.}$ If osmotic pressure and volume of water are converted to moles/cm³ and moles respectively, P_{OS} can be expressed in more general units, i.e., $123 \times 10^{-4} \text{ cm/sec.}$ or $123 \mu/\text{sec.}$

(b) Unequal cell areas in sucrose and water

Kamiya and Tazawa (1956) found unequal rates of water transfer through the cell according to whether the larger area of the cell was in water or sucrose. This was interpreted as a difference in P_{OS} for water movement into and out of the cell. In their smaller cells appreciable internal solute polarisation is produced by quite a small water transfer, hence it is difficult to measure the initial rate of flow accurately. We therefore decided to repeat their experiments, using a very asymmetrical arrangement, on Chara australis, where the polarisation produced by a given transfer of water is much smaller.

The water transfer during transcellular osmosis in a cell with first the larger area in water and the smaller area in 0.2 M sucrose and then vice versa is shown in Fig. 5. It can be seen that the rates were in the ratio of about 1.2:1 when the ratio of the areas was 5:1. This and six other measurements led to an apparent ratio of the osmotic permeabilities in the inward and outward directions of 1.4 ± 0.1 ,

* If P_{OS}^I is the permeability for inward water movement and P_{OS}^{II} is the permeability for outward water movement it can easily be seen from appropriate modifications of equations (3) and (4) that

$$\frac{P_{OS}^I}{P_{OS}^{II}} = \frac{(A_1/A_2) - \frac{(\frac{dv}{dt})_1}{(\frac{dv}{dt})_2}}{\left[(A_1/A_2) \frac{(\frac{dv}{dt})_1}{(\frac{dv}{dt})_2} \right] - 1} \quad (a)$$

where A_1/A_2 is the ratio of the areas, $(\frac{dv}{dt})_1/(\frac{dv}{dt})_2$ is the ratio of initial rates of water flow in the two cases outlined above.

when the sucrose concentration was 0.2 M.

V. DISCUSSION

The Osmotic Permeability

The present experiments with Chara aust. cells lead to a mean value of $5.6 \mu^3/\mu^2 \cdot \text{min. atmos.}$ or $123 \mu/\text{sec.}$ for P_{os} . This is similar to the value obtained by Kamiya and Tazawa (1956) for Nitella flexilis, i.e., $7 - 18 \mu^3/\mu^2 \cdot \text{min. atmos.}$ The value of P_{os} obtained by Palva (1939) for Tolypellopsis was $24 \mu/\text{sec.}$ This was obtained from measurements of the rate of change of the reduced weight of a cell when osmotic water movement was taking place and is not likely to be very accurate.

From the experiments with very unequal areas of the cell in sucrose and water, it would appear that the permeability to water moving into the cell is 1.4 ± 0.1 times greater than the permeability to water moving out of the cell. Kamiya and Tazawa (1956) found a value of 2.66 for the ratio of these two permeabilities but inspection of their fig. 8 suggests that the rapid polarisation of sap solutes led them to overestimate this ratio. They consider this difference in permeability to be real, but it is not necessary to assume a real difference in permeability to account for the effect, for the system is not symmetrical in the two cases, i.e., when sucrose is at the larger end and water at the smaller, and vice versa. From equation (2) it can be seen that the turgor pressure is much lower when the sucrose is surrounding the larger end than when it surrounds the smaller end. Another source of asymmetry arises from the fact that when the smaller end is in sucrose, the velocity of water flow through the area in sucrose is much greater. This leads to a greater concentration of solutes at the internal surface close to the tonoplast and to a greater dilution of the sucrose outside the cell wall and, particularly, in the wall spaces. This effect is not nearly so pronounced when sucrose is at the

larger end. The overall effect is to reduce the osmotic gradient more when sucrose is at the short end and, with it, the apparent permeability.

We are satisfied that this effect of the increased velocity of water flow is an adequate explanation of the apparent difference in permeability for water moving in and out. This conclusion was reinforced by two further observations. The unequal areas experiment was repeated with a more dilute sucrose solution (0.1 M, which would halve the velocity of water flow) and the ratio of the apparent permeabilities was substantially decreased. Also, using equal areas, P_{os} was constant for osmotic gradients up to 8 atm. (cf. Fig. 4, Table 1) but was apparently reduced with greater gradients, i.e., at increasing velocities of flow.

We conclude, therefore, that there is no difference between the permeabilities for water moving in and out. The apparent difference arises because the osmotic gradients are different from what they are assumed to be in calculating P_{os} .

The osmotic permeability of other plant cells has been measured by plasmolysis and deplasmolysis (e.g. Levitt, Scarth and Gibbs, 1936; Mercer and Clark, unpublished) and the values found were of the order $1 - 10 \mu^3/\mu^2 \cdot \text{min} \cdot \text{atmos.}$ However, Mercer and Clark found that P_{os} of isolated (tonoplasts + vacuoles) was up to ten times that of isolated protoplasts. This suggests that the resistance to water movement is not all in the tonoplast (which is demonstrably differentially permeable) but may be in a plasmalemma, cell wall or the cytoplasmic layer. With Kamiya and Tazawa, therefore, we stress that the observed permeability constant does not necessarily refer to a particular membrane, in plant cells.

The Diffusional Permeability

An attempt was made to measure the rate of exchange of D_2O with ordinary

cell water by direct measurement of the change in reduced weight of the cells as $D_2O + H_2O$, of density about 1.05, exchanged with a large volume of H_2O around the cells. This concentration of D_2O (about 50%) had no apparent effect on the cells as evidenced by normal protoplasmic streaming. However, for several reasons it was impossible to place reliance on the rates of equilibration so measured:

(i) It is not known whether the external medium is "stirred" or "unstirred"*. Vigorous stirring can cause a marked increase in the rate of equilibration.

(ii) If P_d is similar to P_{os} , i.e., of the order of $100 \mu/\text{sec.}$, it is clear that the equilibration of a cylinder of diameter 1.0 mm. will be rate limited by the internal diffusion of D_2O up to the cell "membrane". This is apparent when it is considered that a diffusional permeability of $100 \mu/\text{sec.}$ would allow a half-time for D_2O/H_2O equilibration of 2 sec. across the surface of such a cylinder, whereas a cylinder of H_2O of diam. 1 mm. equilibrates with a half-time of 6.9 sec. (stirred, Harris, 1956, p.95) or 19.5 sec. (unstirred, Crank, 1956, p.29) assuming a coefficient of self-diffusion of $2.3 \times 10^{-5} \text{ cm}^2\text{sec}^{-1}$ at 19°C (Wang, 1954). Even if P_d were of the order of $10 \mu/\text{sec.}$ it would be difficult to separate the effect of a differentially permeable barrier from that of slow internal diffusion. A comparison of rates of equilibration of cells with those of 2 per cent agar rods in the same partially stirred condition has revealed very similar rate constants: the half-time of equilibration was about 50 sec. It is therefore impossible to measure the diffusional permeability of these cells in this manner.

* Hicks, personal communication, has pointed out that a density difference of 0.05 between the D_2O/H_2O mixture appearing at the periphery of the cell and the bulk phase is equivalent to a temperature difference of 110°C between the two, and therefore there is some stirring because of convection.

These considerations also apply to the experiments made by Wartiovaara (1944) with Tolypellopsis and those of Nevis (1958) with squid nerves of diam. c.0.4 mm. Neither of these authors took into account the effect of internal diffusion. However, Paganelli and Solomon (1957) showed that, with red blood cells, where the surface area to volume ratio was very much greater, internal diffusion was not rate limiting.

Comparison between P_{os} and P_d

Several workers have interpreted the difference between P_{os} (often termed filtration permeability P_f in the literature) and P_d as due to the presence of pores in a membrane controlling water movement. In these schemes water is envisaged as passing by "bulk flow" along the pores under the osmotic pressure difference, whereas during diffusion a labelled water molecule has to move the full length of such a pore by thermal agitation before it appears in the external medium.

If the pore diameter is greater than about 15\AA , it is probably reasonable to associate P_{os} with hydrodynamic flow according to Poiseuille's law or some suitable modification of it (Pappenheimer, 1953), and hence assign an approximate pore size from the ratio P_{os}/P_d . Such an analysis is probably valid for water flow through such membranes as frog skin and blood capillary walls and possibly for the cell membrane of the frog ovarian egg.

However the normal cell membrane is rather impermeable, and the tonoplast very impermeable, to small ions; this indicates that the pores must be quite narrow, in fact roughly equal to the diameter of a water molecule. An osmotic gradient would then result in a moving file of water through the pores and Harris (1956, p.37) and Hodgkin & Keynes (1955) have shown that this situation leads to a lower value for the tracer flux (which gives P_d) than for the real

flux. Thus the discrepancy between P_{os} and P_d for water movement across cell membranes which are relatively impermeable to small ions is analogous to the discrepancy between the ion permeabilities of say, squid nerve, as measured by membrane conductivity and tracer experiments.

A general quantitative treatment is needed to describe the condition where the pore diameter is greater than the diameter of a water molecule. With plant cells, where the resistance to water movement cannot necessarily be ascribed to a single membrane, the situation is yet more complex.

VI. ACKNOWLEDGEMENTS

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IONIC RELATIONS OF CELLS OF CHARA AUSTRALIS

I. ION EXCHANGE IN THE CELL WALL ¹

by

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Summary

Measurements of ion exchange were made between isolated cell walls of Chara australis and an external solution. Comparison between intact cells and cell walls showed that nearly all the easily exchangeable cations are located in the cell wall. The wall is shown to consist of "water free space" (W.F.S.) and "Donnan Free Space" (D.F.S.); the concentration of indiffusible anions in the D.F.S. is about 0.60 E/l. This finding is contrary to past suggestions that the D.F.S. is in the cytoplasm of plant cells.

The time course of cation exchange, measured with the aid of the radioactive isotopes ²²Na and ⁴⁵Ca, is shown to be complex. The cation exchange could be analysed into fractions with characteristic half-times ranging from about 100 sec. for the "fast" fraction of Na exchanging with ²²Na in the wall to many minutes for the slower fractions.

The "fast" fraction of both Na and Ca exchange is shown to be diffusion limited by a "stationary" film of the order of 100 μ thick outside the wall. It is suggested that the slowing of the remainder of the exchange is due to a combination of steric hindrance in micropores <100 Å in diameter in the wall, together with an electrostatic retardation in the electric double layers of the D.F.S.

I. INTRODUCTION

Recent studies of the ion exchange processes in cells of Nitellopsis (MacRobbie and Dainty, 1958) have revealed three processes separable on the basis

¹ Aust. J. Biol. Sci. - in press.

of characteristic exchange half-time. These were, with respective half-times for K^+ exchange,

1. exchange with "free space" ions ($\sim 1''$),
2. exchange with "non-free space" ions, (~ 1 hr) said to be cytoplasmic,
3. exchange with vacuolar ions, ($\sim 10^3$ hr).

The present study is an attempt to characterize in detail the free space exchange in internodal cells of the alga Chara australis R.Br. v. nobilis.

Several articles have lately stressed the probability that the "Donnan Free Space" (D.F.S.) (Briggs, Hope and Pitman 1958) is located in the cytoplasm with a rather larger "Water Free Space" (W.F.S.) contributed by the cell wall spaces and intercellular spaces (see Briggs and Robertson, 1957; Briggs, 1957). The possible contribution of the cell wall to the initial cation exchange, which is now widely regarded as adjustment of a Donnan system (Briggs, Hope and Pitman, 1958; Middleton and Russell, 1958) has not been explored, although Sutcliffe (1957) states that the cellulosic components of the cell contributed very little to the initial ion exchange in his experiments with disks of red beet.

Earlier experiments (Hope and Stevens, 1952; Hope, 1953) sometimes quoted as evidence for including the cell cytoplasm in the free space have been rightly criticised (Briggs, 1957) as being inadequate, sometimes for the wrong reasons (Levitt, 1957). Further, the considerations of Walker (1957), whose experiments with Nitella point to the existence of a plasmalemma at the surface of the cell which hinders ion exchange in the cytoplasm, suggest that the situation should be reconsidered. The present material was chosen because of the possibility that various phases of the cell could be studied separately. This first paper discusses the exchangeable Na and Ca, and the iodide and mannitol free spaces, (I.F.S., M.F.S.) of the cell wall and the kinetics of the movement of these ions and mannitol between the cell wall and external solution.

II. EXPERIMENTAL METHODS

(a) Material

Strands of *Chara australis* v. *subulata* were collected from ponds and sub-cultured in a polythene lined concrete tank in tap water with a pond mud base.

Several weeks were removed to a the following

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(b) Experiment

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1.5% Na₂CO₃

nodes were in

being bathed in inactive solution, frequently changed. After a certain time the

cell was tightly sealed and placed in a glass dish and bathed with a rubber

squeezer placed over one end: see Fig. 1. The reactivity was then eluted into

aliquots (10 ml for liquid counting, 2 ml, subsequently for solid counting)

of inactive A.P.W. by sucking up liquid from the dish and expelling it, repeated

many times in a given time interval. By this method the liquid can be kept

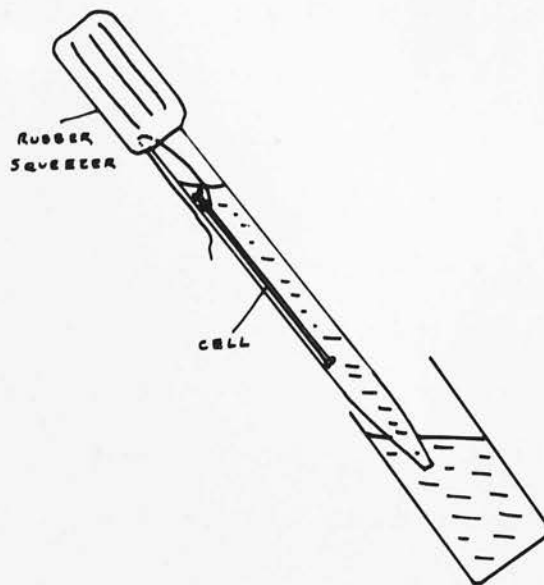


FIG. 1

ELUTION OF CELLS
= WALLS of *CHARA*.

II. EXPERIMENTAL METHODS

(a) Material

Strands of Chara australis v. nobilis were collected from ponds and sub-cultured in a polythene lined concrete tank in tap water with a pond mud base. Several weeks before they were needed for experiment individual strands were removed to a glass aquarium and placed in an artificial pond water (A.P.W.) of the following composition:

NaCl	-	1 mE/l.
CaCl ₂	-	0.5 mE/l.
KCl	-	0.1 mE/l.

This corresponded roughly to the ion content of the field pond water.

Individual internodal cells were cut from a strand and placed in petri dishes in A.P.W. Cells in this condition survived many weeks with normal cytoplasmic streaming and appearance, and with the normal low rate of exchange of sap ions.

The cells were usually 4 - 7 cm long and 1 - 1.5 mm in diameter. The third, fourth or fifth cell from the apex of the strand was chosen if possible.

(b) Experimental procedures

After periods of equilibration of up to 7 days in A.P.W. cells were placed in an A.P.W. of the same composition but labelled with the appropriate tracer ion, i.e., $^{22}\text{Na}^+$, $^{45}\text{Ca}^{++}$, $^{131}\text{I}^-$ or $^{36}\text{Cl}^-$. In experiments with internodal cells the nodes were kept free of radioactivity by means of perspex stocks, the nodal few mm being bathed in inactive solution, frequently changed. After a certain time the cell was lightly blotted and placed in a drawn-out glass pipette and a rubber squeezer placed over one end: see Fig. 1. The radioactivity was then eluted into aliquots (10 ml for liquid counting, 2 ml, subsequently dried, for solid counting) of inactive A.P.W. by sucking up liquid around the cell and expelling it, repeated many times in a given time interval. By this method the liquid medium was kept

stirred. The amounts of activity in the aliquots after various times were determined using conventional Geiger tubes and scalers, or the scintillation counter described below.

In experiments using ^{131}I as a tracer to determine anion free space* the liquid aliquots were made alkaline and some solid sodium thiosulphate added, to prevent oxidation of the I^- and subsequent loss to the atmosphere.

Isolated cell walls were obtained by cutting the nodes from whole cells and scraping gently to remove the contents. The possibility that some cytoplasm remained in such preparations must be kept in mind since some authors consider the spaces between the cell wall microfibrils to contain some interpenetrating cytoplasm. The time course of exchange in walls was measured in the same way as for the cells described above. When the total amount of an ion was to be measured the activity of a cell wall was often counted directly by drying it on a planchette. The specific activity of the solution with which the wall had been in equilibrium was then determined by counting a sample of it, usually $20\mu\text{l}$, which was dried together with a segment of inactive wall. Self-absorption and geometry complications were thus minimised.

The "Mannitol Free Space" of cells and walls was measured by equilibrating the free space with 5 mM mannitol solution which was made radioactive by adding $1\mu\text{C/ml}$ of ^{14}C -1 labelled mannitol. After careful blotting, the activity was eluted into 2 ml aliquots of distilled water or A.P.W. The radioactivity was then determined in a scintillation counter of special design, as follows. The liquid samples were dried on sheets of white mica 20 mm x 45 mm x 20μ thick and

* ^{36}Cl is available at such low specific activities that it could be used only at rather higher than physiological concentrations.

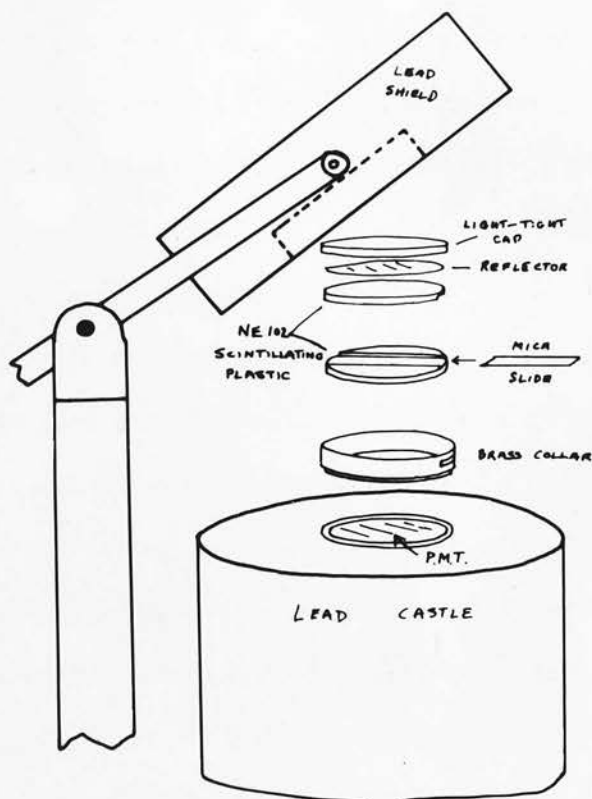


FIG. 2
SCINTILLATION COUNTER

placed between two slabs of scintillating plastic* each 3 mm thick and 50 mm in diam. These were optically coupled to a type 6097 photomultiplier tube. A counting efficiency of approximately 22 per cent for "infinitely thin" samples was obtained with the optimum setting of E.H.T. and discriminator bias, at which the background was usually 80 c/min. This set-up is shown in Fig. 2.

III. RESULTS

(a) Comparison of exchange in intact cells and walls

The time rate of loss of radioactivity from a cell which had been soaked 24 hr in $^{22}\text{Na}^*$ A.P.W. is shown in Fig. 3(a). The activity, on a logarithmic scale, remaining in the cell at various times is plotted against time. (The basis of the use of the log: linear plot is discussed later.)

It is seen that there is a quick loss of activity followed by a much slower one. The cell wall was then isolated and treated in the following way:

1. Soaked for a period of 2 days in inactive A.P.W.,
2. Soaked for 2 days in $^{22}\text{Na}^*$ A.P.W.,
3. Blotted and the activity eluted into A.P.W. as before.

Figure 3(b) shows the time course of the release of the exchangeable Na of the wall. After the initial fast exchange the difference between (a) and (b) (25000 C/min) was found to be in the vacuole of the intact cell. If the extrapolates of the straight line portions are subtracted from the respective total activities, the "quickly exchangeable Na" in intact cell and wall are as shown in Fig. 4 (a) and (b).

The amounts of Na are very similar (104000 c/min in the cell and 102000 c/min in the wall), as are the rates of the exchange ($t_{1/2} = 6.3$ sec for the cell and

* NE102 - Nuclear Enterprises.

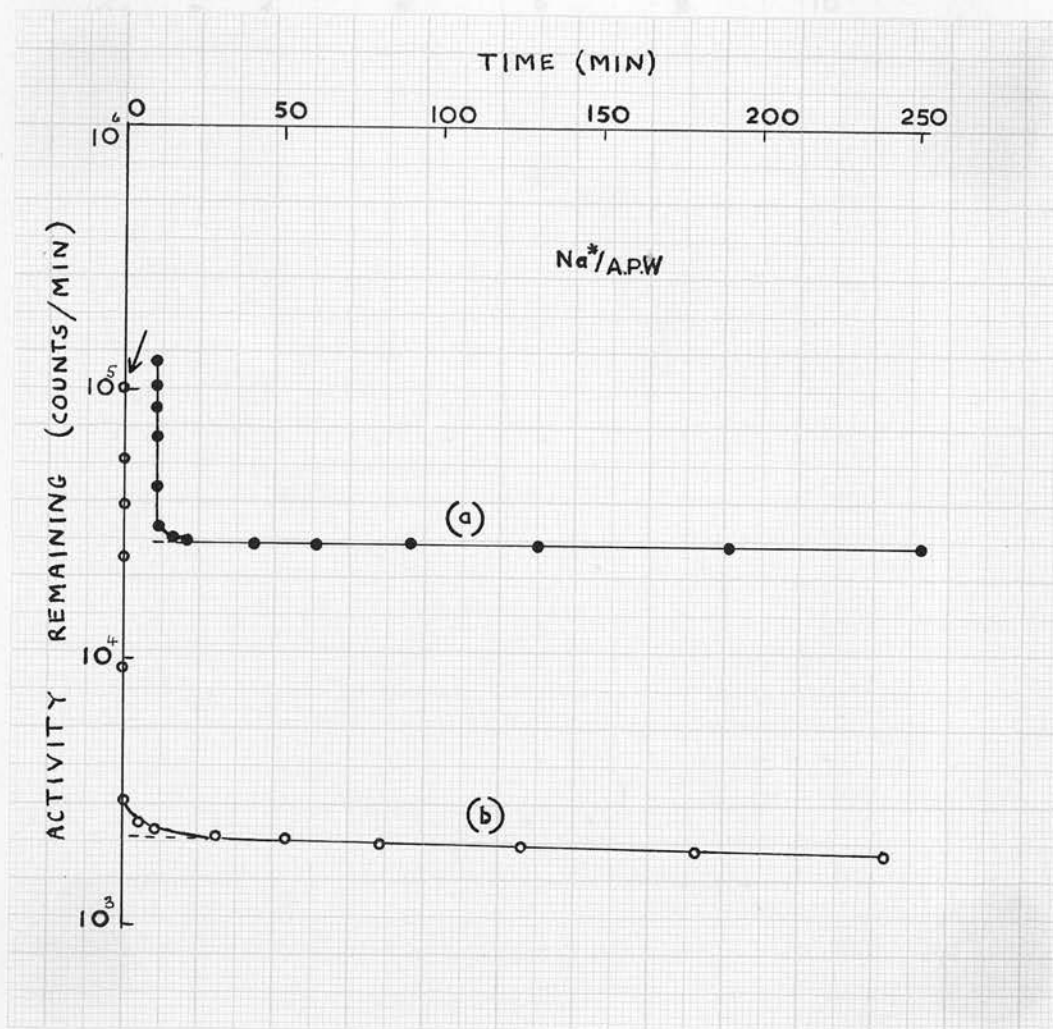


Fig. 3: $Na^*/A.P.W.$

(a) Time course of loss of radioactivity from a cell which had been soaked 24 hr. in ^{22}Na A.P.W.

(zero of time scale shifted to right for clarity)

(b) Time course of loss of radioactivity from isolated cell wall of same cell after two days soaking in ^{22}Na A.P.W.

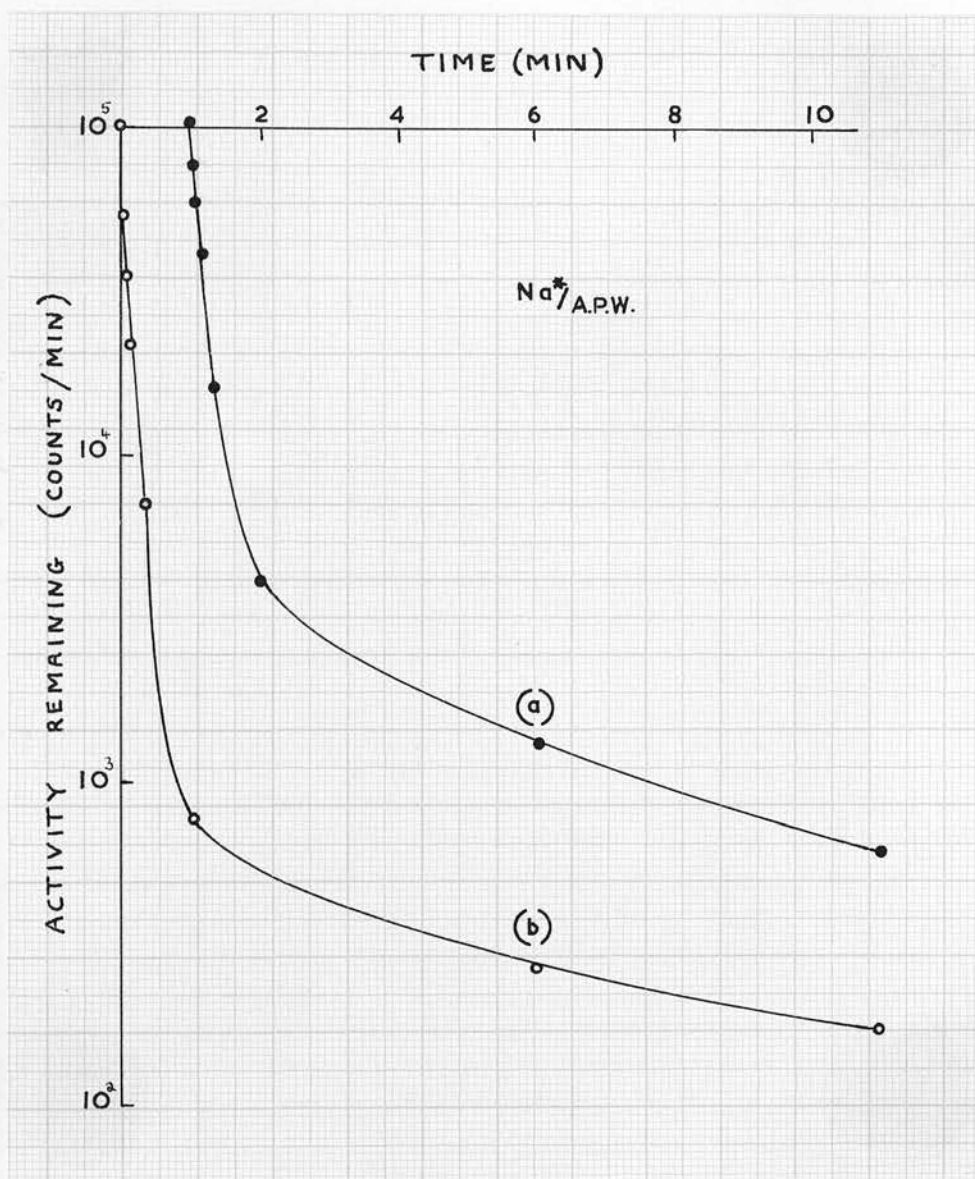


Fig. 4: Na*/A.P.W. Fast fractions.

(a) The curve of 3(a) after subtracting very slow fraction.
Whole cell.

(b) The curve of 3(b) after subtracting very slow fraction.
Cell wall.

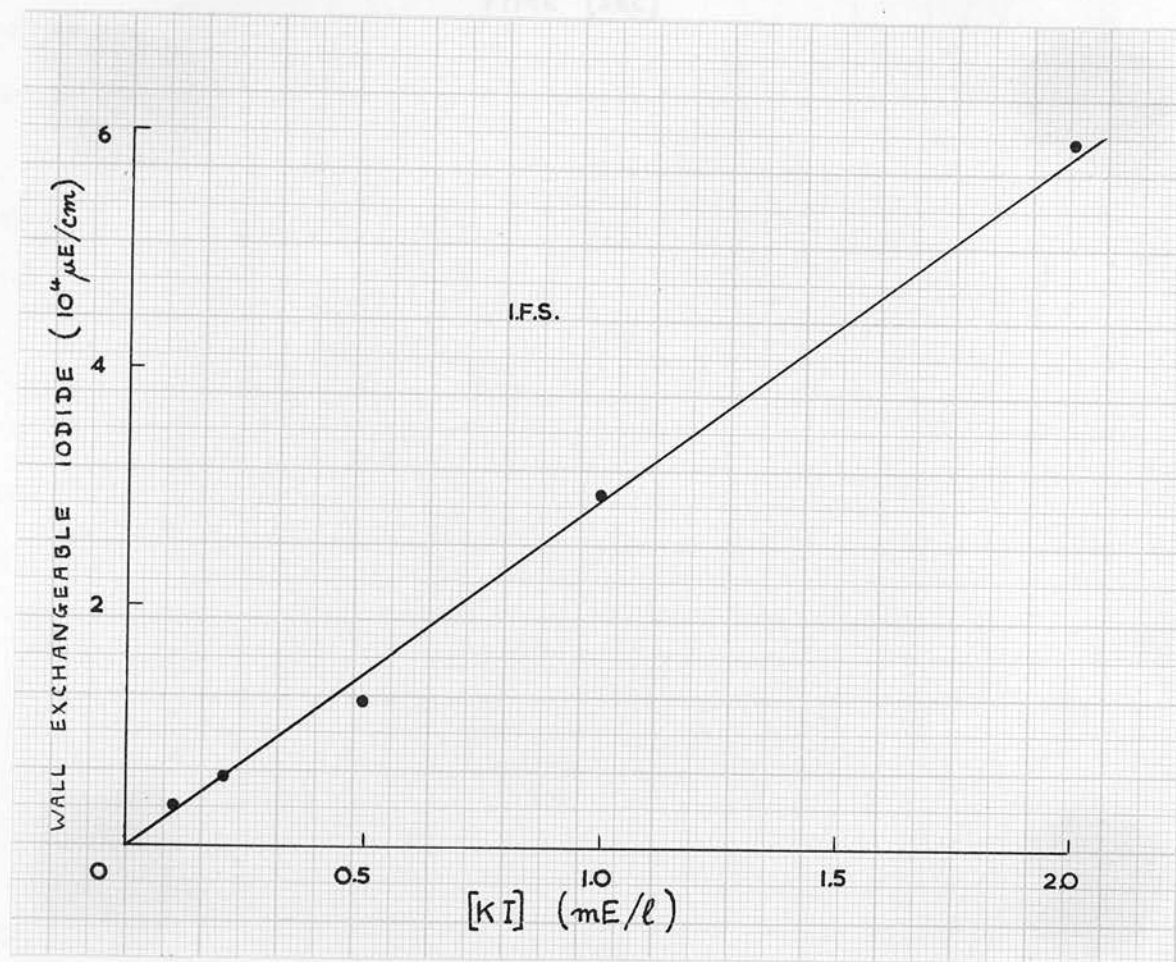


Fig. 5: I.F.S.

The amount of iodide in the cell wall as a function of external concentration.

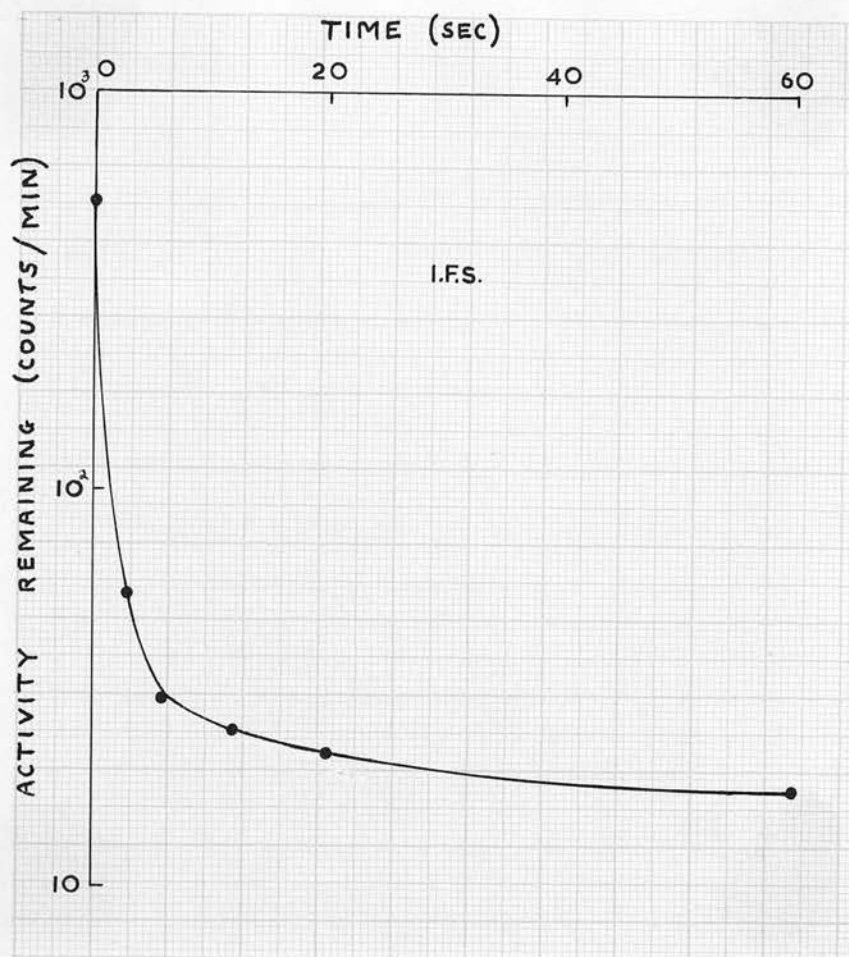


Fig. 6: I.F.S.

Time course of loss of radioactivity from a cell wall which had been previously soaked for about an hour in KI (10meq./l.).

5.6 sec for the wall). The amount of quickly exchangeable Na was in each case 0.03 μ E. The volume of the cell was 75 μ l.

Similar results were obtained using Ca* A.P.W., i.e., the intact cell and wall contained similar amounts of fairly quickly exchangeable Ca. However, the amount was greatly in excess of the exchangeable Na and the exchange rate much slower.

Though it is probable that there are some quickly exchangeable ions further in than the cell wall, it is clear that the majority are located in the latter and that the wall warrants a separate study.

(b) Anion exchange in the cell wall

In Figure 5 the amount of exchangeable I is plotted as a function of concentration. In the pretreatment walls were cut into cm segments and 1 cm from each wall put into the following solutions.

1 mE/l.	CaCl ₂	+	0.1 mE/l.	KI
2 "	"		0.2 "	"
5 "	"		0.5 "	"
10 "	"		1 "	"
20 "	"		2 "	"

for 2 days and for 2 hours into the corresponding solution labelled with ^{131}I . The wall segments were blotted in the standard way and the activity eluted into 10 ml and counted in a liquid counter. Since the amount of exchangeable I in equilibrium with various concentrations of anion is directly proportional to that concentration we are dealing here with exchange with a "Water Free Space" (see Briggs, Hope and Pitman 1958) or "outer space" (Epstein 1955). The mean volume of the I.F.S. in this experiment was 0.29 μ l/cm length of wall.

The rate of equilibration of the wall I with external KI (10 mE/l.) is very rapid, as shown in Fig. 6. A small quantity of I remains after 1 min but most

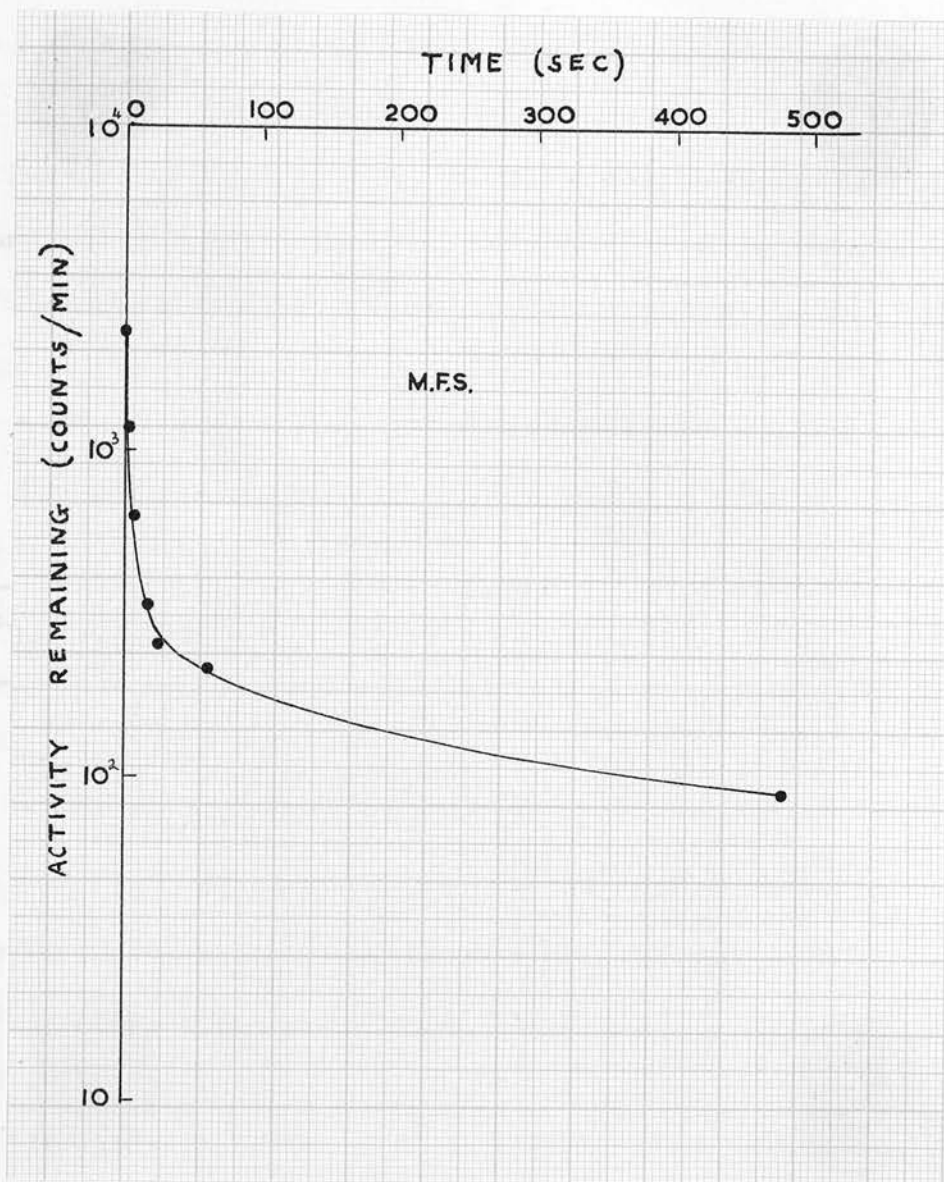


Fig. 7: M.F.S.

The course of loss of radioactivity from a cell wall which had been previously for about an hour in C¹⁴-labelled Mannitol (5mM./l.).

TABLE 1

Expt. no.	I.F.S. (μl)	M.F.S. (μl)	$t_{1/2}$ (sec)	Blotted wt. (mg)	Length (cm)
1	2.2	5.5	1.2 3.5	6.5	6.4
2	1.35	-	1.0	4.1	5.3
3	1.1	-	0.7	3.0	5.0
4	1.45	-	-	-	5.0
5	-	3.6	-	4.6	5.1
6	-	3.2	4	5.0	6.0
7	-	2.6	3	-	6.5
8	-	3.0	2.5	-	-

of it leaves the wall with a half-time of about 0.7 sec. This wall had a surface area of 1.68 cm^2 and a blotted weight of 3.045 mg. Thus, assuming a mean density of 1.1 for such a wall, its thickness would be 16.5μ . The I.F.S. was $1.1 \mu\ell$ ($0.2 \mu\ell/\text{cm}$) which is less than the volume of the wall water which would be about $0.75 \times \text{F.Wt.} = 2.3 \mu\ell$. This fact and the fact that the amount of exchangeable cation is greatly in excess of the exchangeable anion (about $1 \mu\text{E}$ as opposed to $2.4 \times 10^{-3} \mu\text{E}$ with the wall in equilibrium with A.P.W.) suggests that the wall contains a Donnan phase with a high concentration of indiffusible anions. Since the concentration of these is at least of the order of $1 \mu\text{E}/2-3 \mu\ell$, i.e., 0.3 - 0.5 E/ ℓ , the concentration of diffusible anion in the D.F.S. would be expected to be extremely small. Thus the I.F.S. is probably separate from the D.F.S., i.e., the wall is not a homogeneous Donnan phase.

Table 1 summarises the data on anion exchange and I.F.S. of walls.

(c) The Mannitol Free Space of cell walls

The release of labelled mannitol from walls previously soaked in it, into distilled water took place as shown in Fig. 7, a typical experiment. As with iodide, some label remained after 5 min but most of the mannitol diffused from the wall with a half-time of 3 sec. The M.F.S., which is taken to correspond to the whole volume of the wall water penetrated by a molecule of this size, including the D.F.S., was $5.5 \mu\ell$ in this experiment. Table 1 also contains a summary of the M.F.S. of some walls, together with the half-times of equilibration.

(d) Cation exchange in the cell wall

In all the following experiments cell walls were pretreated to try to saturate the native anionic groups with either Na or Ca alone, by soaking several days in NaCl or CaCl_2 solutions, frequently renewed. Then followed an equal period in labelled solution and elution into aliquots of inactive solution as described above. In what follows "Na*/1 Na", etc., means an experiment in which

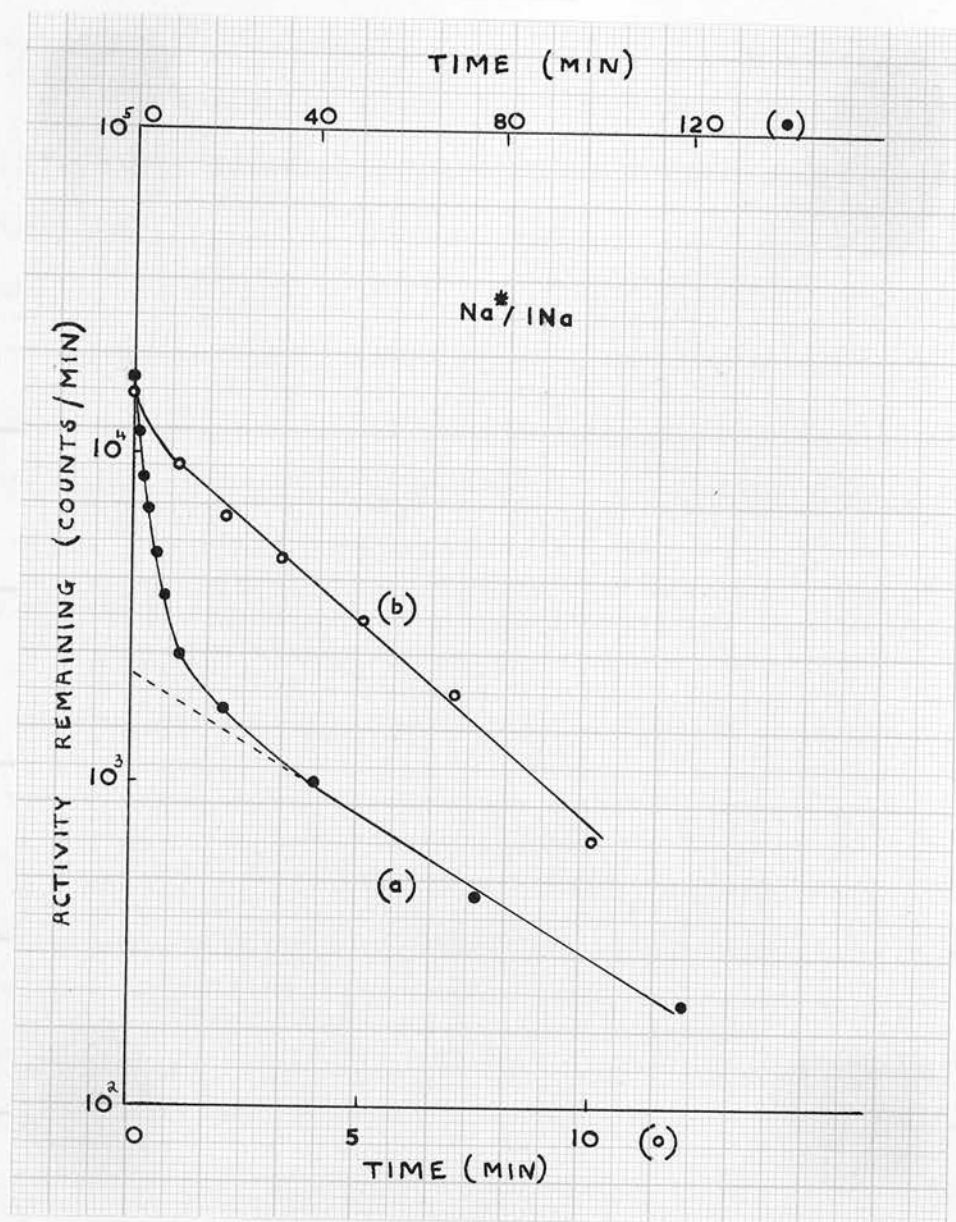


Fig. 8: $\text{Na}^*/1\text{Na}$

(a) Time course of loss of radioactivity from a "Na " cell wall into inactive NaCl (1 meq./l.).

(b) Curve of "fast " component of 8(a), after subtracting "slow" component.

TABLE 2

(1) Mean amounts of exchangeable Na in isolated Chara cell

walls, and mean half-times of isotopic exchange.

Expt.	Total exch. Na(μ E/cm)	Fast Fraction		Other Fractions		No. of observ- ations
		% of total exchanged	$t_{1/2}$ (sec)	%	$t_{1/2}$ (min)	
Na*/1Na	0.12 ± 0.01	75 ± 10	116 ± 13	24 ± 9 1	25 ± 9 (500)	4
Na*/2Na	0.12	70	(170)	26 4	40 (270)	1
Na*/5Na	0.13 ± 0.01	96 ± 3	20 ± 4	3 ± 3 1	(3) (130)	3

labelled sodium was exchanged for Na of concentration 1 mE/l in the external solution.

(i) Na exchange - Fig. 8 shows a $\text{Na}^*/1 \text{ Na}$ experiment. The total exchangeable cation was $0.225 \mu\text{E}$ in a wall segment 2 cm long. The curve of log (radioactivity remaining) vs. time has been divided into 2 parts by extrapolation of the tail of the curve (Fig. 8(a)) to the Y-axis and subtraction of this extrapolate from the total. A very small fraction of the activity ($10^{-3} \mu\text{E}$) remained in the wall after 120 min. The removal of this is not shown on the figure.

An appreciable fraction of the total activity, i.e., $0.028 \mu\text{E}$, exchanged from the wall with a half-time of 34 min. In Fig. 8(b) the activity after subtraction of the "slow" component is plotted, on a log basis, against time. The resulting graph is nearly linear with time, indicating a single exchange rate (see discussion). An amount of $0.195 \mu\text{E}$ exchanged with a half-time of 144 sec. While we do not hold the view that two (or more) discrete "compartments" are necessarily involved in Na exchange in the cell wall, the above procedure does enable characteristic half-times to be ascribed particularly to the fastest exchange, which depend only slightly on the way in which the total curve is divided up. The conclusion from this experiment is that while some (usually the greater part) of the Na is exchangeable with a characteristic half-time of the order of 100 sec for 1 mE/l Na, the remainder is much slower to exchange, surprisingly so in view of the fact that the walls are 16μ thick on the average.

Table 2 summarises the half-times for Na^*/Na exchange, at various concentrations. In some experiments, three segments 2 cm long of the same wall were put into different concentrations of inactive solution; in others, separate walls were used. The half-times are inversely proportion^{al} to the concentration within the accuracy of the experiments. The figures in brackets are very approximate, due to the small amounts of cation being measured, or doubtful due to some other

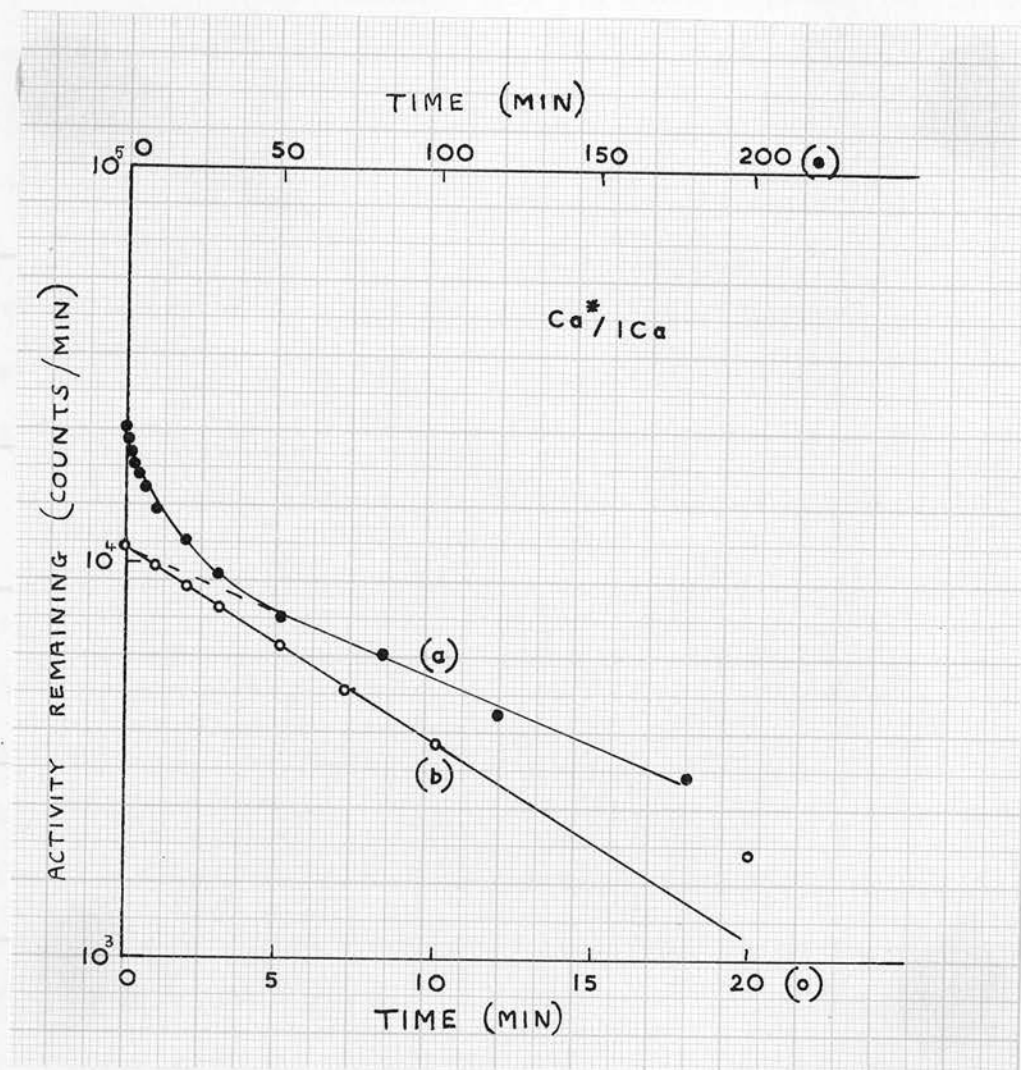


Fig. 9: $\text{Ca}^*/1\text{Ca}$

(a) Time course of loss of radioactivity from a "Ca" cell wall into inactive CaCl_2 (1 meq./l.).

(b) Curve of "fast" component of 9(a), after subtracting "slow" component.

TABLE 3

Mean amounts of exchangeable Ca in isolated Chara cell

walls and mean half-times for isotopic exchange.

Expt.	Total exch. Ca ($\mu\text{E}/\text{cm}$)	<u>Fast Fraction</u>		<u>Other Fractions</u>		No. of observ- ations
		% of total exchanged	$t_{\frac{1}{2}}$ (sec)	%	$t_{\frac{1}{2}}$ (min)	
$\text{Ca}^*/0.5\text{Ca}$	0.14	66	1110	34	345	2
$\text{Ca}^*/1\text{Ca}$	0.20 ± 0.01	52 ± 8	321 ± 16	39 ± 6 10 ± 2	54 ± 13 (800)	4
$\text{Ca}^*/2\text{Ca}$	0.17	73	180	24 3	25 (700)	1
$\text{Ca}^*/5\text{Ca}$	0.20 ± 0.01	87 ± 3	84 ± 16	8 ± 3 5 ± 1	22 ± 4 (550)	4
$\text{Ca}^*/10\text{Ca}$	0.20	93	22	3 4	9 (270)	1
$\text{Ca}^*/1\text{Na}$	(0.2)	26	18000	74	15 days	1

experimental error.

(ii) Ca exchange - Table 3 contains the results of Ca^*/Ca experiments and of one Ca^*/Na . The isotopic exchange of Ca is somewhat slower than that of Na, for the same concentration of the inactive (eluting) medium. A typical experiment is plotted in Fig. 9. The characteristic half-times of the fast fractions, which are almost unambiguous with regard to the splitting up of the total curve, are inversely proportional to the external Ca concentration.

A further feature of both sets of results is that the amount of cation in the fastest exchanging fraction tends to increase as the concentration of eluting solution is increased.

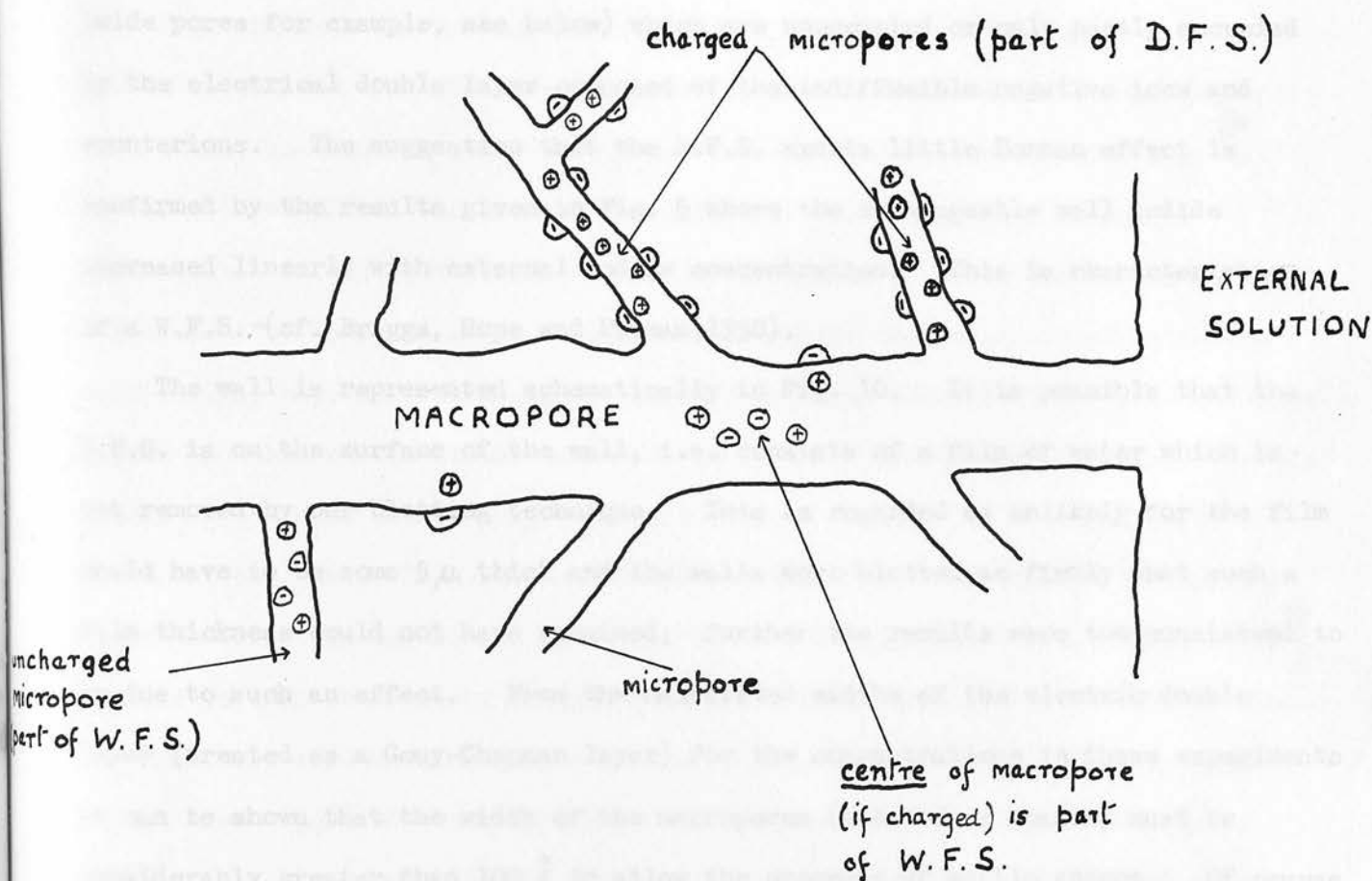
IV. DISCUSSION

The evidence given above, on the nature of the "free space" of Chara cell walls, can be summarised and discussed as follows.

Mannitol probably diffuses into all the wall water since the M.F.S. is approximately numerically equal to 75 per cent of the blotted weight (Table 1) and the dry weight of similar walls was found to be 25 per cent of the blotted ("fresh") weight.

The I.F.S., 35 per cent of the blotted weight, is less than the M.F.S. (Table 1). This immediately suggests some exclusion of mobile anions from part of the wall by fixed negative charges, i.e. by a Donnan system. However, the exclusion is not as great as would be expected if the complete wall were a homogeneous Donnan phase; for, from the total exchangeable cation, the average concentration of the fixed negative charge is about $0.3 \mu\text{E}/\mu\text{l}$ of wall water and this would result in a mobile anion concentration of about $4 \times 10^{-6} \mu\text{E}/\mu\text{l}$ whereas the measured mobile anion concentration is about $10^{-3} \mu\text{E}/\mu\text{l}$, for an outside concentration of $1 \text{ mE}/\ell$. This must mean that the wall is not a homogeneous

FIG. 10



charged micropore diameters $< 100 \text{ \AA}$

macropore diameters, if charged and

if there are no uncharged pores, $\gg 100 \text{ \AA}$

Donnan phase but consists of a W.F.S. and a D.F.S., i.e. it must contain regions (wide pores for example, see below) which are unoccupied or only partly occupied by the electrical double layer composed of the indiffusible negative ions and counterions. The suggestion that the I.F.S. exerts little Donnan effect is confirmed by the results given in Fig. 5 where the exchangeable wall iodide increased linearly with external iodide concentration. This is characteristic of a W.F.S. (cf. Briggs, Hope and Pitman, 1958).

The wall is represented schematically in Fig. 10. It is possible that the I.F.S. is on the surface of the wall, i.e. consists of a film of water which is not removed by our blotting technique. This is regarded as unlikely for the film would have to be some 5μ thick and the walls were blotted so firmly that such a film thickness could not have remained; further the results were too consistent to be due to such an effect. From the calculated widths of the electric double layer (treated as a Gouy-Chapman layer) for the concentrations in these experiments it can be shown that the width of the macropores (water free spaces) must be considerably greater than 100 \AA to allow the presence of mobile anions. Of course, if the fixed negative groups are mostly confined to the micropore system, then the macropore system need not contain such large diameter pores. Indeed one can envisage a system of pores all of much the same diameter but having various amounts of fixed negative charge per unit area of pore wall. However we will usually assume, for the purposes of this discussion, a macro- and micropore system as illustrated in Fig. 10 and as suggested by Northcote (1958).

Since the wall Ca can exchange completely for Na, and from other evidence that the ion distribution obeys the Donnan distribution equations (see Dainty, Hope and Denby, 1959), it is supposed that the forces between the cations and the exchange sites are purely electrostatic and that there is no chemical selectivity other than that due to electric charge. No chelation or other chemical bonds

are envisaged.

From the mean fresh weight/cm (0.8 mg) and the data in Tables 1, 2 and 3 it can be calculated that the average amount of exchangeable Na was $0.19 \mu\text{E}/\mu\text{l}$ cell wall water, in walls in which an attempt was made to replace all the Ca by Na. In walls in which all the counterions were made Ca, the average amount of exchangeable Ca was $0.32 \mu\text{E}/\mu\text{l}$. This difference is probably due to incomplete replacement of Ca by Na or to incomplete ionization of the indiffusible anions (see Briggs, Hope and Pitman, 1958; Dainty, Hope and Denby, 1959). If the larger figure is taken as the better estimate, the average concentration of indiffusible anions in the wall is $0.32 \mu\text{E}/\mu\text{l}$ of wall water. However, a fraction 35/75 of the total wall water is W.F.S. and therefore the true concentration of the fixed negative charge in the D.F.S. is $0.32 \times 75/40 = 0.60 \mu\text{E}/\mu\text{l}$. This figure is very similar to that obtained by Briggs, Hope and Pitman (1958) for beet storage tissue. These authors were unable to specify the location of the D.F.S. in their tissue, though they suggested the cytoplasm. In the present experiments it is quite certain that the great majority of the quickly exchangeable cations (and therefore the D.F.S.) is in the cell wall* (Figs. 3 and 4).

It is clear from Figs. 3 to 9 that the time course of cation exchange is complex. This complexity and the existence of an appreciable W.F.S. can be explained qualitatively by considering the wall as a system of macropores ($>> 100 \text{ \AA}$ in diameter) with a system of micropores ($< 100 \text{ \AA}$ in diameter) leading into them, or by considering it as an essentially micropore system with pore walls of widely differing charge densities or, of course, as a combination of both ideas. Fig. 10 illustrates the suggested macro- and micropore system. We can roughly identify the macropores (or, in the alternative picture, the uncharged pores) with

* Recent experiments by Pitman (private communication) have shown that in beet tissue, too, the D.F.S. is mostly in the cell wall.

the W.F.S. and the micropores (or, alternatively, the highly-charged pores) with the D.F.S. One would expect parts of the micropore (highly-charged pore) system to be more difficult of access than other parts and, because of the greater hydrated size of the Ca ion and its greater charge, Ca would have greater difficulty than Na in getting in to these parts; thus a greater fraction of the exchangeable Ca would be rate-controlled by diffusion and exchange processes in the cell wall than would be the case for Na; this is borne out by the experimental results which show a more complex exchange curve for Ca than for Na. The fast fraction of the exchange curves is explained later as those fairly accessible counterions which can diffuse, in exchange for similar ions, to the surface of the cell wall faster than they can be removed, to the stirred part of the external solution, by diffusion in a stationary film of solution. The slowly exchangeable ions must therefore have considerable difficulty in diffusing through the micropore (or highly-charged pore) system. This can partly be explained by steric hindrance to diffusion in very narrow pores, but there also seems to be an electrostatic effect. The cations in the electric double layer due to the negatively charged walls have a smaller potential energy than those outside the double layers (this effect will be twice as large for Ca as for Na). The activation energy for diffusion of a cation from inside to outside a double layer will therefore be greater than in free solution, i.e. the diffusion coefficient will be lower. This extra activation energy depends on the electric potential in the double layer. Our results show some experimental support for this idea for, as the external concentration is increased, the mean electric potential in the double layer is decreased and more counterions are enabled to diffuse readily. In Tables 2 and 3 it can be seen that the percentage of total exchangeable cation in the fast fraction increases with increase in external concentration. For Na of 1 mE/l, 75 per cent and for Na of 5 mE/l, 96 per cent exchanges quickly. Overbeek (1956) mentions a retardation of counterions in a Donnan phase.

Returning to the "fast" fraction, i.e. the proportion (50 - 96 per cent depending on concentration) exchanging with a single rate constant, we can reasonably make the assumption that the exchange is diffusion limited (Kitchener, 1957). Now ions have to diffuse through the cell wall and then through a stationary film* (unstirred layer) of external solution, before reaching the stirred solution in which we can assume that the concentration of radioactive isotope is zero. The complete solution of this problem is quite complicated (Grossman and Adamson, 1952), but results can be adequately discussed by considering the two limiting cases in which the loss of the radioactive isotope is rate-controlled by (a) diffusion in a stationary film of external solution, or (b) diffusion in the cell wall itself.

If film control is operative, then the concentration of radioactive isotope in the wall, C , is given by

$$C = C_0 \exp (-D^S t / d \delta k) \quad (1)$$

where C_0 is the concentration in the wall at time $t = 0$, D^S is the self-diffusion coefficient of the ion concerned in the external solution, d is the half-thickness of the flattened wall (it is assumed that the wall is washed on both sides), δ is the thickness of the stationary film and k is the ratio of the concentration in moles/l of cell wall of the ion in the wall to the concentration of the ion in the external solution. Clearly $\log C$ is a linear function of time (thus justifying, in this particular case, the log-linear plotting of the results) and the half-time is given by

$$t_{\frac{1}{2}} = 0.69 \frac{d \delta k}{D^S} \quad (2)$$

For the cations sodium and calcium, k is inversely proportional to the external

* Such a film is better thought of as a boundary layer of laminar flow, i.e. a region where flow in a direction normal to the cell wall surface is either very small or absent. Across such a region mass flow must take place by diffusion (see Bircumshaw and Riddiford 1952).

concentration, for the cell wall is a Debye-Hückel solution with a rather high fixed negative charge. Thus if film control is operative the half-time should be inversely proportional to the concentration.

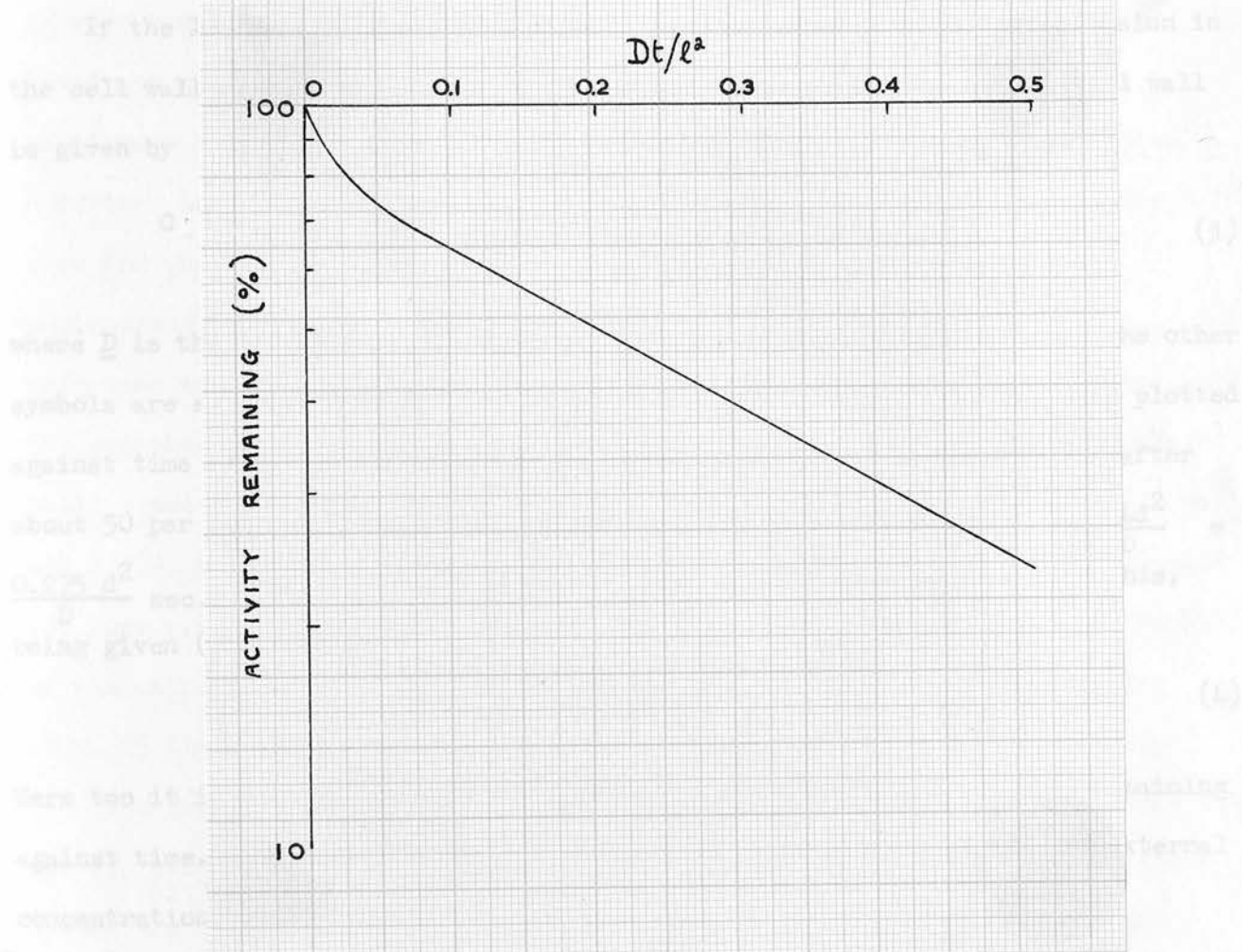


Fig. 11: Time course of loss of radioactivity from a sheet, when the process is rate-controlled by diffusion within the sheet. (See text-equation (3)).

concentration, for the cell wall is a Donnan system with a rather high fixed negative charge. Thus if film control is operative the half-time should be inversely proportional to the concentration.

If the loss of radioactive isotope is controlled by the rate of diffusion in the cell wall itself, then the average concentration of isotope in the cell wall is given by

$$C = C_0 \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-(2n+1)^2 \frac{\pi^2 D t}{4d^2} \right] \quad (3)$$

where D is the effective self-diffusion coefficient in the cell wall and the other symbols are as previously defined or are self-explanatory. When $\log C$ is plotted against time (Fig. 11) the relation is curved at first but becomes linear after about 50 per cent equilibration. From then on C is halved every $\frac{0.69 \cdot 4d^2}{\pi^2 D} = \frac{0.275 d^2}{D}$ sec. The time to reach 50 per cent equilibration is less than this, being given (Fig. 11) by

$$t_{\frac{1}{2}} = \frac{0.197 d^2}{D} \quad (4)$$

Here too it is clearly convenient to plot the logarithm of the activity remaining against time. It is clear that, in this case, $t_{\frac{1}{2}}$ is independent of the external concentration of the ion.

From the data of Tables 2 and 3 it can be seen that the half-times (fast fractions) for ion exchange are inversely proportional to the external concentration within the limits of experimental accuracy. Thus most of the cation exchange between the cell wall and the external solution is, under our experimental conditions, rate-controlled by diffusion in a stationary film of external solution. A further check on this conclusion can be obtained by calculating the value of s , the stationary film thickness, from the experimental data. Taking an average value for d , the cell wall thickness, of 16μ , the average Na concentration in the

cell wall as 130 mE/l of cell wall and the average Ca concentration as 217 mE/l of cell wall, the mean values of δ calculated from equation (2) are 90 μ from the Na results and 106 μ from the Ca results. The order of magnitude of these figures is very reasonable for the stirring conditions used (Bircumshaw and Riddiford 1952; Tetenbaum and Gregor 1954) and the agreement between the two values of the stationary film thickness is strong confirmation of the film-control hypothesis. A further deduction can be made; if the Na exchange is film-controlled, the half-time for the exchange of Na between an untreated cell wall and its normal environment (A.P.W.) can be calculated from the results on "pure Na" walls. This half-time works out to be 10 sec; this is in rough, but adequate, agreement with the experimental values of 5.6 sec (wall with rest of cell) or 6.3 sec (isolated wall) - see Section III (a). The exchange of Ca between the untreated cell wall and A.P.W. proceeds, of course, at the same rate as between Ca-treated cell wall and pure 0.5 mE/l CaCl_2 solution, because Ca is the chief counterion in the D.F.S. of the untreated wall. Thus in the normal cell wall in A.P.W. Ca exchanges about 25 times more slowly than Na.

The average half-time for exchange of iodide between A.P.W. and the cell wall was 1.0 sec and the average half-time for loss of mannitol was 3.3 sec. These values are two or three times larger than expected for pure film control and suggest that the ions or molecules have to pursue rather tortuous paths in the cell wall before reaching the external solution. The comparatively long half-time for iodide exchange is another proof that the I.F.S. (W.F.S.) does not simply consist of a layer of water on the surface of the cell wall. From Figs. 6 and 7 it can be seen that not all the iodide and mannitol leaves the wall with a single rate constant. The small amounts slow to exchange may be hindered in the micropores (mannitol) while the same may apply to iodide provided it penetrates some of the larger micropores.

The results of this study thus indicate that the apparent free space of Chara australis is located in the cell wall and comprises both a water free space and a Donnan free space; the exchange of ions, particularly cations, between the free space and external solutions of 'physiological' concentrations is mainly rate-limited by diffusion in a stationary film of external solution. Presumably the free space in Nitellopsis obtusa, studied by MacRobbie and Dainty (1958), is also entirely confined to the cell wall; they identified in this species an apparent free space, a protoplasmic non-free space and the vacuole as three separate compartments, but were unable to say whether or not part of the protoplasm might be in the apparent free space compartment. A recent study by Diamond and Solomon (1959) on 'Intracellular potassium compartments in Nitella axillaris' is in general agreement with the results of MacRobbie and Dainty (1958) and, in addition, proves that the potassium free space is entirely in the cell wall, which constitutes a Donnan system. They have further proved that the cell wall, the protoplasm and the vacuole are, kinetically, 'in series'.

In our experiments on Chara australis it has so far not been possible to distinguish unambiguously a protoplasmic non-free space in the time course of exchange of Na or Ca in an intact cell. This is partly because of the complex nature of the cation exchange in the cell wall, but studies with ^{42}K - when it becomes available in Australia - should resolve this difficulty. In the past the cytoplasm has been included in the free space, notably by Briggs (1957), Briggs and Robertson (1957) and Briggs, Hope and Pitman (1958); others have considered that a plasmalemma exists at the surface of the cytoplasm and this controls ion transport into the cytoplasm (Levitt 1957; Walker 1957; MacRobbie and Dainty 1958). It would appear from the present study that the free space, including the Donnan Free Space, of Chara australis is located entirely in the cell wall which must contain substances, presumably 'pectins', which can ionise to give rise to fixed

(Donnan) anions (see further Dainty, Hope and Denby, 1959). The protoplasm also, of course, contains indiffusible anions but it does not seem to contribute to the Donnan Free Space; the reason for this may lie in the control of ion transport into the protoplasm by a plasmalemma.

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Notes on water permeability

The original aim of the work described in the paper by Dainty and Hope (1959) on "The Water permeability of Cells of Chara australis" was to compare two water permeability coefficients: that characterising net water transfer due to an osmotic gradient with that characterising (isotopic) water transfer under conditions of osmotic equilibrium. It is said that under the action of an osmotic gradient water is transferred across^a membrane by mass-flow whereas in an isotopic water experiment the transfer is entirely by diffusion; since the membrane will, in general, have quite a different "resistance" to these two types of transfer, a measurement of P_{os} (osmotic permeability coefficient) and P_d (diffusional permeability coefficient) gives information about the membrane structure. If, for example, it is assumed that the water moves through uniform cylindrical pores whose axes are perpendicular to the plane of the membrane, then it can easily be shown that the radius (r) of the pores is given by

$$r = \sqrt{\frac{8V_w \eta D_w}{RT} \cdot \frac{P_{os}}{P_d}}$$

P_{os} and P_d must, of course, be expressed in the same units, e.g. $\text{cm} \cdot \text{sec}^{-1}$.

where V_w is the molar volume (18 cm^3) of water, η is the viscosity and D_w the intrinsic (self) diffusion coefficient of water and R and T have their usual meanings. Solomon and co-workers (1957, 1958), Prescott and Zeuthen (1953) and others have used this concept and the above formula to estimate pore size in the membranes of various erythrocytes and animal egg cells. No measurements have been made on plant cells and we were unable to determine P_d (except to say that P_d is unlikely to be less than one tenth of P_{os}) because the exchange of water between the inside and outside of a cell of Chara australis is rate-limited by diffusion in the vacuole of the cell - as is explained in the paper (Dainty and Hope, 1959).

(If $P_d > P_{os}/10$ and the main barrier to water transport occurs at a single membrane, the pores of this membrane must have a radius less than 12 \AA .)

The first object of these notes is to comment on the concepts involved - mass-flow and diffusion - in water transport, for there has been fierce debate as to whether water moves across a semi-permeable membrane by mass-flow or by diffusion under the action of an osmotic gradient. Chinard (1952) has been the chief proponent of the diffusion hypothesis and he has been opposed by Pappenheimer (1953), Ussing and co-workers (1949, 1953, 1956) and Mauro (1957). There seems little doubt that the experimental evidence (Mauro, 1957, Ussing and Anderson, 1956) is in favour of mass-flow but no convincing theoretical argument for the existence of mass-flow of water has appeared in the biological literature. However a paper by Hartley and Crank (1949) on "Some Fundamental Definitions and Concepts in Diffusion Processes" gives convincing theoretical arguments for the reality of mass-flow. The following paragraphs contain a simplified adaptation and extension of their ideas to the osmotic transfer of water across a semi-permeable membrane.

It is impossible for a diffusion process to take place in a solution without the occurrence of mass-flow, unless the intrinsic (self) diffusion coefficients of the two substances involved are equal. This statement is the crux of the matter and it will now be proved. Consider a solution of a solute (s) in water (w) and suppose there is a concentration gradient $\partial C_s / \partial x$ of solute. Denote the solute and water concentrations, in moles/cm³, by C_s and C_w respectively. Suppose the molar volumes V_s and V_w are independent of concentration; (this assumption is not necessary but it simplifies the mathematics). Denote the intrinsic (self) diffusion coefficients of solute and water by \mathcal{D}_s and \mathcal{D}_w ; i.e. \mathcal{D}_s and \mathcal{D}_w are those diffusion coefficients measured in (isotope) self-diffusion experiments, when there is no mass-flow, or alternatively, they are those diffusion coefficients which are calculable on the basis of Brownian motion or random walk theories.

The sign convention adopted is the ordinary mathematical one: $x, \partial C / \partial x$, increasing from left to right.

The first point to realise is that there cannot be a solute concentration gradient without a water concentration gradient. For, from the definition of concentration and molar volume,

$$V_s C_s + V_w C_w = 1 \quad (1)$$

i.e.
$$V_s \frac{\partial C_s}{\partial x} + V_w \frac{\partial C_w}{\partial x} = 0 \quad (2)$$

Therefore
$$\frac{\partial C_w}{\partial x} = - \frac{V_s}{V_w} \cdot \frac{\partial C_s}{\partial x} \quad (3)$$

Thus in a practical case of diffusion we are always dealing with the simultaneous diffusion of (at least) two substances.

Consider the ordinary experimental arrangement for demonstrating solute diffusion: either a vertical diffusion gradient set up, for example, in a measuring cylinder or - as I shall consider - a horizontal concentration gradient set up in an open trough-like vessel. Consider the transport of solute and water across a section, assumed 1 cm^2 , fixed with respect to the vessel, i.e. fixed so that the volumes of solution on both sides of the section do not change as diffusion proceeds; this section will be referred to as a volume-fixed section and it is, of course, the usual reference section in diffusion experiments. In general

$D_s \neq D_w$ and usually $D_w > D_s$; e.g. for a solution of sucrose in water

$D_w \simeq 2.5 \times 10^{-5} \text{ cm}^2 \cdot \text{sec}^{-1}$ and $D_s \simeq 0.5 \times 10^{-5} \text{ cm}^2 \cdot \text{sec}^{-1}$ at 20°C . Water will thus

have an intrinsic tendency to diffuse faster than solute (for equal concentration gradients) and it is the differing intrinsic diffusion rates which produce (in this case) mass-flow of the solution as a whole. For the volume of substance (solute and water) which tends to cross a section of the solution from left to right in

time dt is given by

$$\phi dt = \left[-V_s D_s \frac{\partial C_s}{\partial x} - V_w D_w \frac{\partial C_w}{\partial x} \right] dt \quad (4)$$

From (2),

$$\phi = (D_w - D_s) V_s \frac{\partial C_s}{\partial x} \quad (5)$$

Thus on the right hand side of the section the volume tends to increase by ϕdt thereby, since the solution is simply confined to an open vessel, causing a transient build up of pressure which, in its turn, will cause mass-flow of the solution to the left. Of course in this case the pressure build-up will be infinitesimal for it is immediately released by mass-flow; but it is quite clear that even in this, possibly the simplest case of diffusion, mass-flow is an inevitable concomitant of the process. We can say that the underlying cause of the mutual diffusion is the Brownian motion of the solute and solvent molecules but, because the intrinsic diffusion rates differ, the actual transport of solute or solvent is the sum of a purely diffusive component plus a mass-flow component. (This situation is somewhat analogous to the diffusion of an ionised salt in water. The ions, in general, have different intrinsic diffusion coefficients and so one of them tends to move ahead of the other. But as soon as it does an electric field is produced by the separation of the charges and this slows down the faster ion and speeds up the slower one. This results in a single diffusion coefficient for the salt.)

Similarly mass-flow results in a single diffusion coefficient for solute and water as determined, in the usual way, from transport across a volume-fixed section. This in itself is impressive evidence for mass-flow and it is proved below. Denote the diffusion coefficients for solute and water with respect to a volume-fixed section by D_s^v and D_w^v , respectively. Since the section is "volume-fixed" there

can be no net transport of volume across the section. (Any tendency towards this is corrected by mass-flow as explained above.) Therefore

$$-V_s D_s^v \frac{\partial C_s}{\partial x} - V_w D_w^v \frac{\partial C_w}{\partial x} = 0 \quad (6)$$

But
$$V_s \frac{\partial C_s}{\partial x} + V_w \frac{\partial C_w}{\partial x} = 0 \quad (2)$$

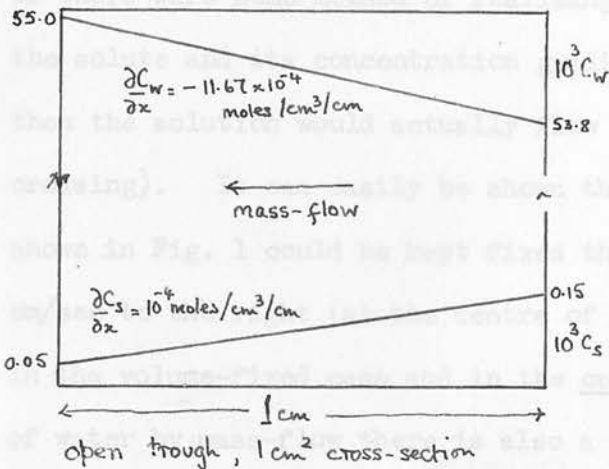
Therefore
$$D_s^v = D_w^v \quad (7)$$

i.e. the diffusion process can be described by a single diffusion coefficient, D^v . Since the intrinsic diffusion coefficients may be very different it is clear that some process other than Brownian motion must be involved in the transport across a volume-fixed section and, from the previous argument, this is mass-flow. (It can be shown that mass-flow makes a major contribution to the flux of solvent across a volume-fixed section, but only a minor contribution to the flux of solute in ordinary solutions. Thus $D^v \neq D_s$ and very different from D_w . Fig. 1 and the accompanying legend gives a numerical example of the various quantities involved in the mutual diffusion of sucrose and water.)

It should now be clear that mass-flow always accompanies diffusion processes. However I have so far only considered the obvious and common case of mass-flow and diffusion in an open system relative to a volume-fixed section. A more interesting case and step nearer to consideration of osmotic water transport is transport of water across a section which is fixed relative to the solute. Across such a section, of course, there is no transport of solute and therefore the diffusion flux, $D_s \partial C_s / \partial x$, must be exactly balanced by the mass-flow flux, $V C_s$; thus there is a mass-flow, relative to the solute, with a velocity given by

$$V = \frac{D_s}{C_s} \cdot \frac{\partial C_s}{\partial x} \quad (8)$$

FIG. 1



Let $\bar{C}_s = 0.1 \times 10^{-3}$ moles / cm³,
and $\frac{\partial C_s}{\partial x} = 10^{-4}$ moles / cm³ / cm.

Now $V_s = 210$ cm³ / mole for sucrose

$D_s = 0.5 \times 10^{-5}$ cm² . sec⁻¹

$D_w = 2.5 \times 10^{-5}$ cm² . sec⁻¹

$V_w = 18$ cm³ / mole

$\therefore \frac{\partial C_w}{\partial x} = -11.67 \times 10^{-4}$ moles / cm³ / cm

and $\bar{C}_w = 54.4 \times 10^{-3}$ moles / cm³.

Mass - flow = $V_s D_s \frac{\partial C_s}{\partial x} + V_w C_w \frac{\partial C_w}{\partial x}$ to the right
 $= +105 \times 10^{-9} - 525 \times 10^{-9}$ to the right
 $= 420 \times 10^{-9}$ cm / sec = ϕ , to the left.

Flux of water (to the right) across volume - fixed section at centre of trough

is given by $-D^v \frac{\partial C_w}{\partial x} = \underbrace{-D_w \frac{\partial C_w}{\partial x}}_{\text{by diffusion}} - \underbrace{\frac{\phi C_w}{22.8 \times 10^{-9}}}_{\text{by mass-flow}}$ moles / cm / sec
 $= +29.2 \times 10^{-9}$

Therefore $D^v = 6.4 \times 10^{-9} / 11.67 \times 10^{-4} = 0.54 \times 10^{-5}$ cm² . sec⁻¹.

Flux of solute (to the right) across volume - fixed section at centre of trough

is given by $-D^v \frac{\partial C_s}{\partial x} = \underbrace{-D_s \frac{\partial C_s}{\partial x}}_{\text{by diffusion}} - \underbrace{\frac{\phi C_s}{0.04 \times 10^{-9}}}_{\text{by mass-flow}}$
 $= -0.5 \times 10^{-9}$

Therefore $D^v = 0.54 \times 10^{-9} / 10^{-4} = 0.54 \times 10^{-5}$ cm² . sec⁻¹.

If there were some method of realising this solute-fixed section, i.e. of keeping the solute and its concentration gradient fixed relative to the walls of the vessel, then the solution would actually flow along the vessel from left to right (x increasing). It can easily be shown that if the sucrose concentration gradient shown in Fig. 1 could be kept fixed there would be mass-flow at the rate of 5×10^{-6} cm/sec to the right (at the centre of the gradient): more than ten times more than in the volume-fixed case and in the opposite direction. In addition to the transfer of water by mass-flow there is also a small transfer by pure diffusion amounting, in the above example, to about one tenth of the transfer by mass-flow.

There is a practical method of realising a solute-fixed section: by confining the solute to one side of a solution by means of a semi-permeable membrane. This case is exactly the same, in principle, as the one discussed in the previous paragraph; therefore mass-flow of water occurs from the water compartment through the semi-permeable membrane into the sucrose compartment. However the semi-permeable membrane does have an influence on the amount of mass-flow; in effect instead of the concentration gradient of sucrose, and its concentration, determining the velocity of the mass-flow, the properties of the membrane determine the velocity of mass-flow which, in its turn, determines the sucrose concentration gradient near the surface of the membrane.

The above discussion, though proving the necessity for mass-flow, is perhaps rather abstract and does not quite get down to a detailed physical picture of the processes involved, particularly for the water transport across a semi-permeable membrane. The following account and calculation of the pressure causing mass-flow will, I hope, be even more convincing; to the best of my knowledge this calculation, though rough, is quite new though it is based on the old Eyring theory of rate processes (Glasstone, Laidler and Eyring, 1941).

Consider a pore, in the semi-permeable membrane, of cross-section A cm² as

pure water

$A \text{ cm}^2$

$\lambda \text{ cm}$

solute concentration = C_s

solute mole fraction = x_s

shown in Fig. 2. (The pore does not have to be cylindrical, straight or uniform.)

We can think of the solute end of the pore as constituting a sharp division between pure water in the pore and solution to the right of the pore. Let the average separation of the water molecules be λ cm, so that we can consider a layer of pure water molecules just inside the pore and, λ cm away, a layer of water plus solute molecules. By definition the solute molecules cannot pass into the pore. If n_L and n_R are the number of water molecules per cm^3 of pure water and solution respectively, then the numbers of water molecules in the two layers are $n_L A \lambda$ and $n_R A \lambda$. The molecular picture of the process of diffusion is as follows; each molecule oscillates within a confined volume and when, due to the random movements of its neighbours, a "hole" opens near by, it will jump into the hole, i.e. diffuse, and of course leave a vacancy behind it. Thus each molecule has a certain probability $k dt$ of making a jump (of the order of λ cm) in time dt in a given direction; i.e. k is the number of jumps a given molecule makes per second. Therefore the number of molecules jumping from layer L to layer R in time dt is $n_L A \lambda \cdot k dt$ and the number of molecules jumping from layer R to layer L in time dt is $n_R A \lambda \cdot k dt$. Thus the net number of vacancies created in time dt in layer L is $(n_L - n_R) A \lambda \cdot k dt$. These vacancies persist for a time of the order of $\tau = \lambda / \bar{c}$ where \bar{c} is the mean velocity of the molecules ($\bar{c} \approx \sqrt{RT/M} = 4 \times 10^4 \text{ cm. sec}^{-1}$); τ can be called the relaxation time for the process and, clearly, if we put $dt = \tau$ then $(n_L - n_R) A \lambda \cdot k \tau$ is the average number of vacancies always present in layer L. Thus layer L has a lower density than the pure solution further back in the pore and therefore a lower pressure; this is the source of the pressure which drives solvent - by mass-flow - through the pores and into the solution on the right-hand side. This pressure can be calculated as follows; the fractional decrease in density is given by

$$\frac{(n_L - n_R) A \lambda \cdot k \tau}{n_L A \lambda} = x_s \cdot k \tau$$

where x_s is the mole fraction of the solute in the right-hand compartment. The decrease in pressure in layer L can be calculated from the experimental value of the compressibility of water, which is 50×10^{-6} per atmosphere.

Thus
$$\frac{dv}{v dp} = \frac{dp}{\rho dp} = 50 \times 10^{-6} \text{ atmos.}^{-1},$$

therefore
$$dp = \frac{1}{50 \times 10^{-6}} \cdot \frac{dp}{\rho} = \frac{x_s \cdot k\tau}{50 \times 10^{-6}} \text{ atmospheres.}$$

As I have already explained $\tau = \lambda / \bar{c}$; $\lambda \approx 10^{-8} \text{ cm}$ and $\bar{c} = 4 \times 10^4 \text{ cm/sec}$, therefore $\tau = 2.5 \times 10^{-13} \text{ sec}$. k , the probability of jumping in a given direction, can be derived from the viscosity of water; according to Glasstone, Laidler and Eyring, 1941, $k \approx RT/\eta V_w = 1.3 \times 10^{11} \text{ sec}^{-1}$. $k\tau$ is thus equal to 3×10^{-2} ; this implies that a water molecule makes about 30 oscillations before it makes a jump to fill a "hole". Thus

$$dp = \frac{3 \times 10^{-2}}{50 \times 10^{-6}} \cdot x_s = \frac{3 \times 10^{-2}}{50 \times 10^{-6}} \cdot \frac{C_s}{18},$$

i.e. $dp \approx 30 \cdot C_s \text{ atmos.}$; C_s is in moles/litre.

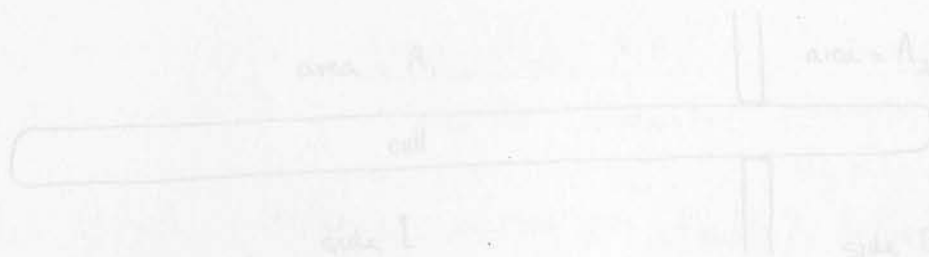
Therefore, according to this simple purely kinetic theory, mass-flow of water must occur and is "driven" by a pressure difference of about $30 \cdot C_s$ atmospheres. The thermodynamic theory of osmotic pressure of dilute solutions gives this pressure as $RT \cdot C_s \approx 24 \cdot C_s$ atmospheres, but it has nothing to say on the mechanism of transport. An accurate kinetic theory of liquids, which we do not have, would presumably give the same result as the thermodynamic theory.

Thus, given a semi-permeable membrane with a water-filled pore structure, it can now be shown that the diffusion of water from the end of the pore causes a

pressure, approximately equal to $RT \cdot c_s$, which produces mass-flow down the pore; the magnitude of the flow is determined by the pore dimensions. (It should be stressed that osmosis only takes place by bulk flow when the solutions are separated by a semi-permeable membrane with solution filled pores. If the solutions are separated by a vapour gap or by a layer of liquid, through which the water can only pass by dissolving in the liquid, then bulk-flow cannot occur and the transport of water is entirely by diffusion. Whatever kind of membrane separates the solutions water transport will proceed from the dilute to the concentrated solution and, in a suitable experimental arrangement, will build up the classical hydrostatic pressure (osmotic pressure) $RT \cdot \Delta c_s$.)

This effect of the increased velocity of water flow is an adequate explanation of the apparent difference in permeability for water moving in and out. The following paragraphs give the detailed theory of the effect of water flow on apparent water permeability, discuss the results of Emlen and Tammes in the light of the theory and also consider various other phenomena to which the theory is applicable.

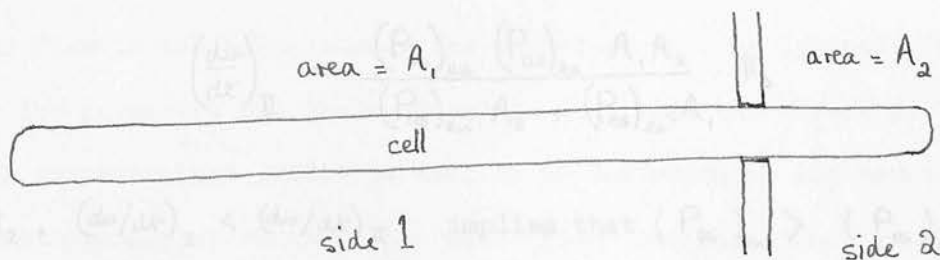
The basic result to be explained is the following (refer to the figure below).



If sucrose solution, of osmotic pressure Π_s , is put on side 2 and distilled water on side 1, there will be an initial rate of flow of water given by $(J_w/at)_i$. If the experiment is repeated with the same sucrose solution on side 2 and

Another objective of the work reported in the paper on "The Water Permeability of cells of Chara australis" was investigation of the apparent polar permeability of water reported by Kamiya and Tazawa (1956). They deduce from transcellular osmosis experiments on Nitella flexilis using an asymmetrical arrangement that the endosmotic permeability constant is 2.66 times the exosmotic permeability constant. Such a suggestion is so contrary to what might be expected on physico-chemical grounds that it must be looked at extremely critically. Dr. Hope and I also found an apparent difference between the permeability constants for water moving into and out of the cell, though not as great as that of Kamiya and Tazawa. However, as we state in the paper, "we are satisfied that this effect of the increased velocity of water flow is an adequate explanation of the apparent difference in permeability for water moving in and out". The following paragraphs give the detailed theory of the effect of water flow on apparent water permeability, discuss the results of Kamiya and Tazawa in the light of the theory and also consider various other phenomena to which the theory is applicable.

The basic result to be explained is the following (refer to the figure below).



If sucrose solution, of osmotic pressure Π_o , is put on side 2 and distilled water on side 1, there will be an initial rate of flow of water given by $(dw/dt)_1$. If the experiment is repeated with the same sucrose solution on side 1 and

distilled water on side 2 there will be an initial rate of flow of water given by, say, $(dv/dt)_{II}$. The experimental observation is that $(dv/dt)_I < (dv/dt)_{II}$ (both ours and that of Kamiya and Tazawa). Now if the endosmotic and exosmotic permeability coefficients are equal (to P_{OS}) then

$$\left(\frac{dv}{dt}\right)_I = \left(\frac{dv}{dt}\right)_{II} = P_{OS} \cdot \frac{A_1 A_2}{A_1 + A_2} \cdot \pi_0 \quad ; \quad (1)$$

(the equality arises because of the symmetry of the term $A_1 A_2 / (A_1 + A_2)$.)

Since $(dv/dt)_I \neq (dv/dt)_{II}$ Kamiya and Tazawa assume that $(P_{OS})_{en} \neq (P_{OS})_{ex}$; in this case the following equations apply:

$$\left(\frac{dv}{dt}\right)_I = (P_{OS})_{en} \cdot A_1 \cdot (\pi_i - p_r) \quad (2)$$

$$\left(\frac{dv}{dt}\right)_{II} = (P_{OS})_{ex} \cdot A_2 \cdot (\pi_0 - \pi_i - p_r) \quad (3)$$

where π_i is the initial internal osmotic pressure and p_r is the turgor pressure.

Hence

$$\left(\frac{dv}{dt}\right)_I = \frac{(P_{OS})_{en} \cdot (P_{OS})_{ex} \cdot A_1 A_2}{(P_{OS})_{en} A_1 + (P_{OS})_{ex} A_2} \cdot \pi_0 \quad (4)$$

Similarly, for case II (sucrose at large end - 1),

$$\left(\frac{dv}{dt}\right)_{II} = \frac{(P_{OS})_{en} \cdot (P_{OS})_{ex} \cdot A_1 A_2}{(P_{OS})_{en} A_2 + (P_{OS})_{ex} A_1} \cdot \pi_0 \quad (5)$$

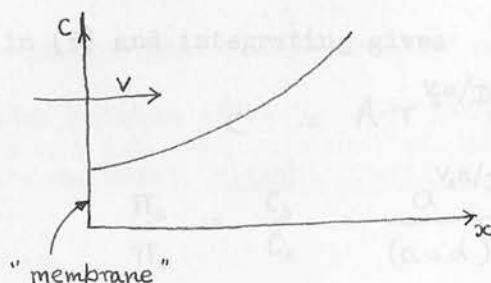
Since $A_1 > A_2$, $(dv/dt)_I < (dv/dt)_{II}$ implies that $(P_{OS})_{en} > (P_{OS})_{ex}$, i.e. the water permeability is greater when water is moving into the cell than when it is moving out.

Thus Kamiya and Tazawa interpret the fact that $(dv/dt)_I \neq (dv/dt)_{II}$ by a difference in the term P_{OS} in equation (1) in the two cases. We suggest that there is a much simpler explanation, which entirely fits the facts, namely a

difference in the driving force in the two cases.

In case I (sucrose at small end - 2) water flows in through the large area A_1 and out through the small area A_2 . Therefore the velocity of flow is greater through the small area into sucrose, than through the large area. This water flow produces (as will be proved below) concentration gradients of the solutes; thus the solute concentration just inside the vacuole at the large end will be less than π_i/RT and just inside the vacuole at the small end it will be greater than π_i/RT ; and the sucrose concentration just outside the small end will be less than π_o/RT . Because the velocity of flow across the small area is greater (about five times greater in our experiments) than the velocity of flow across the large area, the decrease in the driving force $(\pi_o - \overline{\pi_i} - \rho_r)$ will be greater at the small end. In case II (sucrose at the large end - 1) there will be a much smaller decrease in the driving force because the velocity of flow across the area in sucrose is much less than in case I. Thus, qualitatively, one can see immediately a possible reason for the smaller flow of water in case I than in case II; it will now be proved that there is also an excellent quantitative fit with our results and this effect could explain those of Kamiya and Tazawa. (In the following the concentration gradients produced by the water flow in the vacuole near the tonoplast will be ignored; they tend to increase the asymmetry but their effect is less than the effect on π_o because the internal concentration gradients tend to be destroyed by the vacuolar stirring caused by protoplasmic streaming; the water flow also has a smaller effect on the internal solutes because their diffusion coefficients are relatively large.)

At first, for simplicity, consider a one-dimensional case.



In the steady state there will be no net transfer of solute across any section (perpendicular to x); the amount of solute supplied by diffusion across the section plus the amount carried by the flow of water must therefore be zero.

Thus From equation (1)

$$-D \frac{\partial c}{\partial x} + vc = 0 \quad (6)$$

And, therefore, $c = A e^{vx/D}$, where A is a constant. (7)

From the cell dimensions ($A = 0.065$ cm, $A_1 = 2.1$ cm², $A_2 = 0.42$ cm²). From the

The water flow therefore produces a concentration gradient of solute and therefore decreases the concentration of solute (sucrose) at the external boundary of the cell. At some distance d from the surface (which can be expected to be of the order of 100-1000 μ - the unstirred boundary layer, see the second paper of Dainty and Hope) the concentration will be the bulk concentration $c_o (= \pi_o/RT)$. Hence from equation (7), the concentration of solute at the external surface of the cell is given by

$$c_s = c_o e^{-vd/D} \quad (8)$$

and the effective driving force (RTC_s) can be calculated.

However, we are dealing with flow through the surface of a cylinder when equation (6) has the same form, i.e.

$$-D \frac{\partial c}{\partial r} + vc = 0 \quad (9)$$

but V now varies with r and is given by equation (10), in which V_s is the velocity of flow at the surface and a is the radius of the cylinder:

$$V = V_s a/r \quad (10)$$

Substituting for V in (9) and integrating gives

$$c = A r^{V_s a/D} \quad (11)$$

thus

$$\frac{\pi_s}{\pi_o} = \frac{c_s}{c_o} = \frac{a^{V_s a/D}}{(a+d)^{V_s a/D}} \quad (12)$$

where d is the thickness of the boundary layer. This equation (12) will now be applied to our results in order to determine the value of d and to see whether it is reasonable or not.

From equation (1)

$$\frac{(dv/dt)_I}{(dv/dt)_II} = \frac{(\pi_s)_I}{(\pi_s)_II} = \left(\frac{a}{a+d} \right)^{\frac{a}{D}(V_s)_I} / \left(\frac{a}{a+d} \right)^{\frac{a}{D}(V_s)_II} \quad (13)$$

From the cell dimensions ($a = 0.065$ cm, $A_1 = 2.1$ cm², $A_2 = 0.42$ cm²), from the graph of Fig. 5 in the paper $\left[(dv/dt)_I = 0.017 \mu\text{l/sec} \text{ , } (dv/dt)_{II} = 0.021 \mu\text{l/sec} \right]$ and from $D = 5 \times 10^{-6}$ cm²sec⁻¹ for sucrose, $(V_s)_I = 4.0 \times 10^{-5}$ cm.sec⁻¹ , $(V_s)_{II} = 1.0 \times 10^{-5}$ cm.sec⁻¹ and $a/D = 1.3 \times 10^4$ cm⁻¹sec. Therefore

$$\frac{0.017}{0.021} = \left(\frac{0.065}{0.065 + d} \right)^{0.52} / \left(\frac{0.065}{0.065 + d} \right)^{0.13}$$

and hence d is approximately 400μ . This is a very reasonable figure for the unstirred boundary layer around the cell under the mild stirring conditions which existed during the experiment and thus it is considered that the change in the driving forces is the correct explanation of the fact that $(dv/dt)_I \neq (dv/dt)_{II}$. It is quite unnecessary, at least in our experiments, to invoke the rather dubious concept of polar permeability. This conclusion is reinforced by other observations. It can be deduced from equation (13) that if π_o is decreased from 4.9 atmospheres to 2.5 atmospheres,

$$\frac{(dv/dt)_I}{(dv/dt)_{II}}$$

is increased from 0.83 to 0.91. This is in agreement with our observations and is contrary to the concept of polar permeability, unless indeed one makes the additional assumption that polar permeability depends on the velocity of flow.

The discrepancy between $(dv/dt)_I$ and $(dv/dt)_{II}$ is much greater with Kamiya and Tazawa's material, Nitella flexilis, even at a relatively low

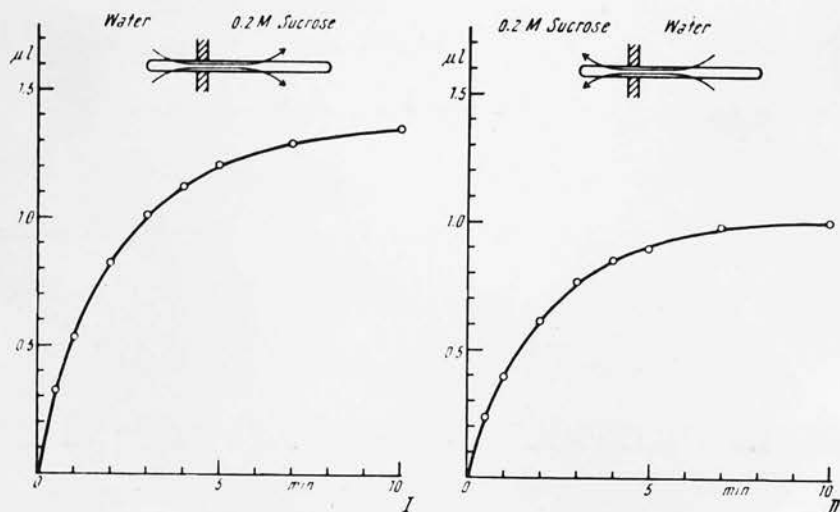


Fig. 8. Two forward osmosis curves in one and the same cell under the same osmotic pressure difference of 0.2 M. In this preparation, the position of the partition wall is such that the left and right ends of the cell protruding into the compartments are, excluding the length mounted in the partition, 12 mm. and 51 mm. respectively. In (I) water enters the cell at the shorter end (left) and escapes from the longer end (right), while in (II) water moves in the reverse direction.

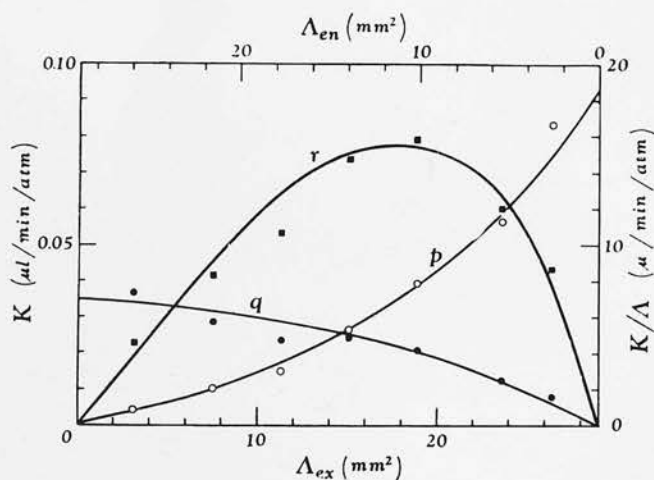


Fig. 9. Squares represent experimentally obtained values of the transcellular water permeability (K) at various positions of the partition wall (left hand ordinate). Open circles show the values of K divided by endosmotic surface area (A_{en}), and solid circles represent the values of K divided by exosmotic surface area (A_{ex}) of the cell. Through extrapolation of the curves p and q to zero surface areas, specific water permeabilities of the cell for endosmosis and exosmosis, k_{en} and k_{ex} , can be obtained. Curve r represents theoretical values of the transcellular water permeability K obtained from formula (44). Temperature: 20° C.

The two relevant figures from Kamiya and Tazawa (1956).

asymmetry - $A_1/A_2 = 31/12$. Their values are $(dv/dt)_I = 0.10 \mu\text{l}/\text{min}/\text{atm.}$, $(dv/dt)_{II} = 0.15 \mu\text{l}/\text{min}/\text{atm.}$, $A_1 = 0.428 \text{ cm}^2$, $A_2 = 0.166 \text{ cm}^2$, $a = 220 \mu$, $\pi_0 = 4.9 \text{ atm}$ (0.2 M sucrose). They calculate, on their assumptions of polar permeability, that $(P_{os})_{en} / (P_{os})_{ex} = 2.66$ [$(P_{os})_{en} = 18.4 \mu^3/\mu^2/\text{min}/\text{atm.}$, $(P_{os})_{ex} = 6.9 \mu^3/\mu^2/\text{min}/\text{atm.}$]. On the assumption that the difference in water flow is due to a decrease in the driving force, I calculate from equation (13) that $d \sim 1 \text{ cm}$! This figure is not so alarming as it looks; in the first place Kamiya and Tazawa's experiment was done under much more stagnant conditions than ours and d might be expected to be much larger; also quite a small error in determining $(dv/dt)_I / (dv/dt)_{II}$ would cause a very large change in the calculated value of d . For example if the ratio $(dv/dt)_I / (dv/dt)_{II}$ were 0.83 instead of 0.67, d would fall to 1 ^{mm}. Such an error of this magnitude and direction, is quite possible for the accuracy of their experiments was much lower than ours and they are likely to underestimate the initial rate of flow - particularly in case I (sucrose at short end) - because of the rapid curvature of the dv/dt vs. t graph before they made their first observation.

Another related observation made by Kamiya and Tazawa is at first sight quite startling; this is that if dv/dt is plotted as a function of either A_1 or A_2 (sucrose at end 2), it reaches a maximum at an asymmetrical position at which $A_2 > A_1$, i.e. the area in sucrose is the larger. They of course interpreted this in terms of different endosmotic and exosmotic water permeabilities, but the phenomenon can equally well be explained by the decrease of the driving force produced by water flow. Equation (1) should be written

$$\frac{dv}{dt} = P_{os} \cdot \frac{A_1 A_2}{A_1 + A_2} \cdot \pi_s \quad (14)$$

and, from (12),
$$\frac{dv}{dt} = P_{os} \cdot \frac{A_1 A_2}{A_1 + A_2} \cdot \pi_0 \cdot \left(\frac{a}{a+d} \right)^{\frac{a}{d}} \cdot \frac{dv/dt}{A_2} \quad (15)$$

Clearly this equation is not symmetrical in A_1 and A_2 and therefore the maximum value of dv/dt will not occur at $A_1 = A_2$. This maximum value can be found by differentiating dv/dt with respect to A_2 and equating $d(dv/dt)/dA_2$ to zero. The result is that the maximum value of dv/dt occurs when

$$1 - \frac{A_2^2}{A_1^2} = P_{os} \cdot \pi_o \cdot \left(\frac{a}{a+d} \right)^{\frac{a}{D}} \cdot \frac{dv/dt}{A_2} \cdot \ln \left(\frac{a}{a+d} \right)^{\frac{a}{D}} \quad (16)$$

Since the R.H.S. < 0 , $A_2 > A_1$, i.e. the maximum occurs when the greater area is in sucrose in agreement with the results of Kamiya and Tazawa. They found the maximum value of dv/dt to be $0.077 \mu\text{l}/\text{min}/\text{atm}$ occurring at $A_2 = 0.18 \text{ cm}^2$ and $A_1 = 0.11 \text{ cm}^2$ (to within 0.01 cm^2); also from their results $P_{os} = 18.5 \mu^3/\mu^2/\text{min}/\text{atm}$. From these figures a value of d can be calculated; the values $A_2 = 0.18 \text{ cm}^2$ and $A_1 = 0.11 \text{ cm}^2$, or anything more asymmetrical lead to impossibly large values of d ; but if we take $A_2 = 0.17 \text{ cm}^2$ and $A_1 = 0.12 \text{ cm}^2$ (which are perfectly possible values according to Fig. 9 in the paper of Kamiya and Tazawa) the value of d , according to equation (16), is approximately 1000μ .

It seems clear that the results of Kamiya and Tazawa can be explained without invoking the need for polar permeability of water, if their experimental techniques and results are examined critically. It is certainly better to explain this asymmetrical water transport by a well-known physico-chemical effect than to accept the results at their face value as denoting polar permeability. If anyone invokes an unlikely concept, the burden of proof is on him and Kamiya and Tazawa - in an otherwise excellent paper - have failed to do this.

Another example of the use of the above-described theory of diffusion in a moving medium occurred in the same experiment. As transcellular osmosis proceeds the rate of flow of water decreases because the internal solutes become polarised and so reduce the driving forces. If there are no forces tending to

depolarise the internal solutes, transcellular osmosis proceeds until the polarisation of the solutes reduces the driving forces to zero; then no further water flow is possible. In practice, instead of stopping, a steady small water flow is finally established which goes on indefinitely.

In an experiment (not described in the paper) in which transcellular osmosis was measured over a long time with 0.05 M sucrose ($\pi_0 = 1.25 \text{ atm}$), the initial rate of flow was $0.35 \mu\text{l/min}$ and after a few minutes dv/dt became constant at $0.047 \mu\text{l/min}$ and remained at this value indefinitely. Thus since the driving force producing the flow of $0.35 \mu\text{l/min}$ was 1.25 atm , the driving force producing the final steady flow must have been 0.17 atm ; therefore an internal solute polarisation of 1.08 atm was finally present during this long continued trans-cellular osmosis. (This corresponds to a salt concentration difference of about 22 mM .) At this steady state there must have been a depolarising effect preventing the full polarisation of 1.25 atm from being reached.

One possible cause of depolarisation is the simple back-diffusion of the internal solutes. If this is the cause then, at the steady state, the back-diffusion of salts must be exactly counterbalanced by the forward transport of salts by mass-flow. That is, if back-diffusion is the cause, the velocity of flow must be related to the concentration gradient by the following equation:

$$D \frac{dc}{dx} = vc$$

i.e. $C = A e^{vx/D}$ where A is a constant.

If the original osmotic pressure of the salts was 7.5 atm , then at the steady state the osmotic pressure at the exosmosis end is $7.5 + .54 = 8.04 \text{ atm}$ and the osmotic pressure at the endosmosis end is $7.5 - .54 = 6.96 \text{ atm}$. Therefore if d is the distance over which the concentration gradient exists we have, from

the above equations,

$$\frac{8.04}{6.96} = e^{\frac{vd}{D}}$$

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The velocity of flow up the vacuole at the steady state is 6×10^{-5} cm/sec; D, for vacuolar solutes, is about 1.5×10^{-5} ; therefore $d = 0.36$ mm. Thus the velocity of mass-flow along the vacuole is adequate to maintain the observed concentration difference over as small a distance as 0.36 mm. The concentration difference is more probably spread over at least a centimetre and the mass-flow should therefore produce a much greater concentration difference; thus back-diffusion is likely to contribute very little to solute depolarisation.

A crude calculation shows that the most likely cause of depolarisation is the stirring of the vacuolar contents by the streaming protoplasm. Using a crude model a streaming velocity of 10μ /sec will produce exactly the amount of depolarisation observed. A direct measurement on the streaming velocity of Nitella translucens gave 65μ /sec.

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passive i.e. purely physico-chemical movement of ions and molecules can and does take place into and out of the protoplasm and vacuole. However there is a formidable diffusion barrier at the tonoplast and, probably, at the plasmalemma and in principle, though not necessarily in practice, a kinetic analysis could distinguish between the physico-chemical adjustments going on outside these barriers and that going on across the barriers. This distinction is easily made with a single cell (Mackintosh and Dainty, 1958.) but can become quite blurred with tissues; hence physico-chemical readjustment with tissues may - on a kinetic basis - include processes which one would normally exclude - or at least put in a different category - when studying readjustment with single cells.

This physico-chemical readjustment of a plant tissue to a new external solution has been discussed in the botanical literature in terms of the "Free space" concept - a concept which has given rise to as much confusion as

When a plant tissue (root, leaf, storage tissue, algal cell, etc.) which has been previously equilibrated with an external solution of a certain concentration, is placed in a different external solution there is a fairly rapid readjustment between the ions and molecules present in the tissue and those in the new external environment. One envisages, that, after a fairly short time in a given external solution, there is physico-chemical equilibrium between ions and molecules in certain parts of the tissue (e.g. intercellular spaces, cell walls,) and those in the external solution. Thus any change in the external solution leads to the setting-up of a new physico-chemical equilibrium.

Of course there will also be changes taking place in the solutes in the vacuoles and, probably, in the protoplasm; it will be assumed, at the moment, that these changes can be distinguished by suitable experiments from those concerned with the readjustment of the purely physico-chemical equilibria. This term - physico-chemical - will be used for those processes in which no energy is supplied by the cells' metabolism. There is some inconsistency in these two sentences because it is quite certain (MacRobbie and Dainty, 1958,) that passive i.e. purely physico-chemical movement of ions and molecules can and does take place into and out of the protoplasm and vacuole. However there is a formidable diffusion barrier at the tonoplast and, probably, at the plasmalemma and in principle, though not necessarily in practice, a kinetic analysis could distinguish between the physico-chemical adjustments going on outside these barriers and that going on across the barriers. This distinction is easily made with a single cell (MacRobbie and Dainty, 1958,) but can become quite blurred with tissues; hence physico-chemical readjustment with tissues may - on a kinetic basis - include processes which one would normally exclude - or at least put in a different category - when studying readjustment with single cells.

This physico-chemical readjustment of a plant tissue to a new external solution has been discussed in the botanical literature in terms of the "Free Space" concept - a concept which has given rise to as much confusion as

understanding and it is part of the purpose of this article to try and clarify both the concept and, more importantly, the process of physico-chemical adjustment.

Two definitions of free space may be quoted as illustrating what leading botanists think it is. "Free space describes that part of a plant tissue which is in free diffusion communication with the environment, without permeation barriers." (George Laties, 1959, personal communication.)

"The free space (F.S.) of a cell or tissue is that part into which the solute and solvent from the external solution penetrates readily; this is in contrast with that part of the cell or tissue into which the solvent but not the solute penetrates readily" (Briggs and Robertson, 1957.)

It is clear from these quotations and the remarks in the two first paragraphs that Laties' definition is ideally the better one and is certainly applicable to single cells; but the definition of Briggs and Robertson is the practical definition, at the present time, for plant tissues which contain a large number of cells. For the free space is estimated from the ^{rapid} phase of readjustment of a plant tissue and in a moderately-sized piece of tissue - such as is customarily used in experiments - the time of diffusion through the intercellular spaces and along and through cell walls may well be larger than the time required for an appreciable passive readjustment across the plasmalemma or even the tonoplast. Thus if the definition of Briggs and Robertson is used for free space, the latter may include parts of the tissue not envisaged by Laties' definition, which is certainly more "intellectually" desirable. Laties' definition is certainly the one which will ultimately be used (if the term "free space" has not by then fallen into disuse, for the importance of the concept is only that of helping us to understand and characterise this physico-chemical readjustment.) From these remarks it is clear that free space is not quite the same thing when determined on a single

cell, e.g. on one of the Characeae, as it is when determined on a tissue in the usual kind of experiment.

Thus the free space of a tissue (as opposed to a single cell) is quantitatively a somewhat nebulous concept because one can never be sure which morphological features of the tissue are involved in the rapid phase of passive readjustment. There are also further serious difficulties in its characterisation and these have led to the introduction of the term "apparent free space" (A.F.S.) to describe, operationally, the results of a free space experiment. Briggs and Robertson (1957) define apparent free space as follows: "If U is the estimated amount of free space uptake of a solute by a tissue originally devoid of this solute from a solution of concentration C , then the A.F.S. is U/C ". Clearly the units of A.F.S. are ml./unit weight of tissue and the definition can be put somewhat more instructively as that volume of the tissue which would be occupied, readily, by the solute taken up if the solute were at the same concentration as the external solution. Simple considerations show that the A.F.S. as defined above is not a unique quantity; it varies a great deal with the kind of solute and the type of experiment used. Briggs and Robertson (1957) and Briggs (1957) have explained this point in great detail; it therefore suffices to point out here, without explanation, that very different values will be obtained for the A.F.S. for each of the following typical solutes; carbon dioxide, mannitol, insulin, protein, monovalent cation, divalent cation, monovalent anion, divalent anion, weak acid anion, weak base cation, hydrogen ion, hydroxyl ion, etc. Also quite different values of A.F.S. using the same solute can be obtained by uncritical use of the following experimental methods: conductivity determinations, change in concentration in bathing solution, chemical determination of change in amount of solute in tissue, isotopic exchange, etc. It must not therefore be imagined that A.F.S. is a useless concept; on the contrary important information about the physico-chemical readjustment (which after all is the aim)

is obtained from these very variations in A.F.S. It is useful in fact (Dainty and Hope, 1959,) to replace the term A.F.S. by such expressions as sodium free space, mannitol free space, chloride free space, etc. - terms which are self explanatory.

A.F.S. determinations using small, non-lipid-soluble, neutral solutes such as mannitol give fairly consistent results. In fact the parts of the free space, into and out of which they move, probably comprise the intercellular spaces (if filled with liquid) and parts of the cell wall and may be considered as a simple extension of the external environment and hence in simple diffusion "equilibrium" with it. It is tempting to call this part of the free space the "water free space" (W.F.S.) but this expression has not been used in a consistent manner in the literature and I feel it is better to leave it as e.g. mannitol free space. A further discussion of the so called W.F.S. will be included in the following paragraph on "ion free space".

If a plant tissue which has been equilibrated for some time in distilled water is transferred to a dilute solution of, say, rubidium bromide then there is a fairly rapid uptake, soon completed, of Rb and Br. (It will be assumed that the metabolic uptake can be corrected for, or suppressed by low temperatures or metabolic inhibitors.) It is found that, in general, there is a greater uptake of Rb than of Br and that the extra uptake of Rb (over Br) is due to exchange of Rb for cations already in the tissue. Clearly quite different values will be obtained for the Rb.F.S. and the Br.F.S. Also the Rb.F.S. is very dependent on the pretreatment of the tissue, i.e. whether the tissue has had a long soaking in solutions of monovalent or divalent cations, and even the Br.F.S. is slightly dependent on this pretreatment. It is indeed possible to get values for the cation free space of several ml./g. of tissue and the concept of free space is made absurd by quoting such values. Further, quite different values for the cation free space can be obtained by using cations of different charge. Briggs (1957) and Epstein (1955) have "overcome" this difficulty of definition (which is all

it is) by defining apparent free space (Epstein calls it "outer space") as the anion free space; but even this can vary and, in general, is not equal to, say, the mannitol free space.

However these difficulties of definition are quite unimportant, for consideration of the uptake and exchange of ions is of the greatest value in assessing the properties of the free space of the tissue - the region in which physico-chemical adjustments take place. It is now quite clear, thanks to the penetrating analysis of Briggs (1957), that the free space consists of a region or regions in which both ions of a salt are in equal concentrations - he calls this the water free space (W.F.S.) - and a region or regions in which the cation is in excess, usually in great excess, of the mobile anion - this he calls the Donnan free space (D.F.S.) and it must clearly contain a high concentration of fixed or, at least, indiffusible anions (negative charge). There is little difficulty in suggesting where these spaces are likely to be located in the plant tissue, but there is a great deal of controversy as to where they are located.

The W.F.S., which merely consists of an extension of the external environment, certainly includes the intercellular spaces, unless of course they are filled with air. From recent experiments by Dainty and Hope (1959) part of the cell wall (in Chara australis, about 30%,) must also be considered as being in the W.F.S. Some, e.g. Briggs and Hope, have even considered that the W.F.S. penetrates into the cytoplasm. I think this latter suggestion must be considered unlikely at present, although it is just possible that pinocytosis - if it occurs in plant cells - might give a small "cytoplasmic" contribution to the W.F.S., and similarly an endoplasmic reticulum might be considered to have direct connection with the fluid in the cell wall. Other regions into which the W.F.S. penetrates are the xylem vessels, possibly some of the sieve tubes, and cut and injured cells at the surface of the tissue. In addition any film of solution left at the surface of the tissue by inadequate blotting or centrifugation techniques will be included in the W.F.S. and this

can be quite a large contribution in such tissues as cereal roots (Levitt, 1957). Most of the quoted values of the W.F.S. (or anion free space or outer space) are so large (~ 0.20 ml./g.) that they must include contributions from these latter miscellaneous sources which, to some extent, must be considered artefacts. The only experimental investigation of the site(s) of the W.F.S. known to me is that of Pitman (1958); he estimated from autoradiographs of beet storage tissue that, of the measured 0.20 ml./g. W.F.S., only 0.05 ml./g. is in the intercellular spaces and cell walls; the rest - 0.15 ml./g. - is in the vessels and in the cut and injured cells at the surface of the tissue. Presumably a similar division will be found in other measured values of the W.F.S.

Two possible locations for the Donnan free space (D.F.S.) have been suggested: the cell wall and the protoplasm. Both of these regions, without any doubt, contain indiffusible anions which are a prerequisite for a D.F.S.; the cell wall contains uronic acids, as units in the polysaccharides, not all of whose carboxyl groups are methylated, and it would therefore act like a weak acid ion exchange resin; the protoplasm also contains various kinds of anions - organic phosphates, organic acids such as malic, protein amino-acid side chains such as glutamic acid which will be ionised at the usual protoplasmic pH, and perhaps other special indiffusible anions (as for example the isethionate in squid axons). However it is becoming clear that the major part of the D.F.S. is in the cell wall; indeed it is rather surprising that the extreme view that all the D.F.S. is in the protoplasm was ever adopted. It would be expected, depending on the solute used, that with a piece of plant tissue of the size normally used in free space experiments a small part of the D.F.S. would be in the protoplasm, (see discussion at the beginning of this article). With a single cell, however, all the D.F.S. is in the cell wall (Dainty and Hope, 1959.). At my suggestion Dr.M.G.Pitman of the Cambridge Botany School

has recently measured the D.F.S. of beet tissue and the D.F.S. of undegraded cell wall from the same weight of tissue. He finds that the exchange capacity (a measure of the D.F.S.) of the whole tissue is $11.3 \pm 0.7 \mu\text{eq./g.}$, while that of the cell wall of an equal amount of tissue is $8.9 \pm 0.2 \mu\text{eq./g.}$ (personal communication). Laties (personal communication) has quoted perfect agreement between the exchange capacity of fresh oat coleoptile and that of the cell wall material extracted from it. For the time being then the D.F.S. will be discussed as if it were all in the cell wall but consideration will be given later to the contribution likely to be made by the protoplasm in experiments with lumps of tissue.

It is not necessary to describe here the exchange properties of the D.F.S., how they depend on the "native" counterions and their valency and the valencies of the exchanging cations and anions, on the relative volumes of the D.F.S. and the external solute, on the concentration of the fixed anion and the pH of the external solution, on whether the cations and anions are ions of weak bases or acids, and so on. Nor is it necessary to discuss the pitfalls of the various experimental methods used. All this has been done admirably - albeit a little obscurely - by Briggs (Briggs and Robertson, 1957; Briggs, 1957.). I wish to discuss various points not mentioned by Briggs

Footnote to page -6-.

Dr.D.M.W.Anderson of the Department of Chemistry, University of Edinburgh, is kindly making analyses of the polysaccharides of Chara australis and Nitella translucens. He finds that on a total cell dry weight basis, Chara contains 15% and Nitella 24% uronic acids. (It is an extremely interesting fact, because of the nul action of auxin on Nitella, that none of the uronic acid is methylated.) If it is assumed that about 50% of the total cell dry weight is cell wall, then the above uronic acid contents imply concentrations of 1.5 meq./g. cell wall dry weight for Chara and 2.4 meq./g. cell wall dry weight for Nitella. Since wet Chara cell wall contains 3ml.water/g.dry weight, the mean concentration of fixed negative charge is 0.5 meq./ml.of water. This is about twice the mean concentration found by Dainty and Hope (1959) from measurements of the exchangeable Ca, but in the above calculation the fraction of dry cell weight due to cell wall has been guessed. Further analyses of isolated cell wall are being planned to eliminate this guess.

It is thus quite clear that in Chara australis and Nitella translucens there is a very high concentration of fixed negative charge supplied by the uronic acids.

and particularly to probe more deeply the the concept of D.F.S. and W.F.S. as it applies to cell wall, (for the intercellular part of the W.F.S. is a trivial and obvious extension of the external environment). Also, since Briggs is inclined to the view - quite wrongly in my opinion - that all the D.F.S. is in the protoplasm, I wish to discuss exactly what contribution the protoplasm is likely to make to the D.F.S. in an experimental determination using tissue.

The experimental method of proving that the free space of a cell wall can be separated into a W.F.S. and a D.F.S. has been based (implicitly by others and explicitly by Dainty and Hope, 1959,) on the following theory. Suppose the volume of water in a cell wall is V_{ml} and the amount of fixed anion, assumed "monovalent", in the wall is F moles. Then if this is uniformly distributed throughout the volume V , i.e. there is a uniform D.F.S., the average concentration of fixed anion is F/V moles/ml. Let the external concentration of a uni^{uni}-valent salt be changed from zero to C_0 moles/ml. and suppose the volume of external solution is infinite. Let the externally-supplied cations be of the same kind as the counter-ions in the D.F.S. Then the original concentration of counter-ions will be F/V moles/ml and of co-ions zero; the new/concentrations of counter-ions and mobile anions (co-ions) are found by solving the following equations in which C_i moles/ml and a_i moles/ml are the final concentrations of counter-ions and co-ions. Only the elementary Donnan theory is used in which all activities are put equal to concentrations and pressure terms are ignored; though it would be interesting to consider the effect of the high tensions to which the cell wall is normally subjected under the usual turgor conditions.

$$C_0^2 = C_i a_i \quad (1)$$

$$C_i = a_i + F/V \quad (2)$$

Therefore

$$2a_i = -\frac{F}{V} + \sqrt{\left(\frac{F}{V}\right)^2 + 4C_0^2} \quad (3)$$

hence if $C_o \ll F/V$, the usual situation,

$$a_i \approx C_o^2 / \frac{F}{V} \quad (3a)$$

Similarly
$$2C_i = \frac{F}{V} + \sqrt{\left(\frac{F}{V}\right)^2 + 4C_o^2} \quad (4)$$

and
$$C_i \approx \frac{F}{V} + \frac{C_o^2}{F/V} \quad \text{if } C_o \ll F/V \quad (4a)$$

For most plant cell walls of the type used in free space experiments $F/V \sim 200 \text{ mM/l}$; therefore equations (3a) and (4a) can certainly be applied for values of C_o up to 10 mM/l . In this range the anion uptake is therefore expected to be parabolic (proportional to C_o^2) if all the free space of the cell wall can be treated as D.F.S. Further the magnitude of the anion uptake should be quite small; in fact the W.F.S. should equal $C_o / \frac{F}{V} = V \cdot \frac{C_o}{F}$. These calculations were applied by Dainty and Hope (1959) to the cell wall of Chara australis and the relevant discussion can be found in their paper. Another example which can be used as an illustration (although this is for whole tissue, not just cell wall) is from the beet storage tissue investigated by Pitman (1958). In this case F is about $10 \mu\text{M/g}$ tissue, V about 0.07 ml/g . therefore F/V is $140 \mu\text{M/ml}$. If C_o is $10 \mu\text{M/ml}$, then a_i is expected to be $100/140 \mu\text{M/ml}$ and the anion free space is expected to be $100/140 \times 1/10 \times 0.07 = 0.005 \text{ ml/g}$ tissue. This is ten times smaller than that found and a linear relationship (not a parabolic one) between uptake of anion and external concentration was also found. Thus the free space cannot be wholly a D.F.S. but must comprise a W.F.S. and a D.F.S. i.e. a region in which the anion concentration is equal to the external concentration and one in which it is very much smaller due to exclusion by the fixed negative charge.

An analysis of the nature of an inhomogeneous Donnan system, and its splitting into a D.F.S. and a W.F.S., much deeper than any that have appeared

in the botanical literature is possible. Some suggestions were made by Dainty and Hope (1959) and in the following paragraphs an attempt is made to extend these considerably giving a detailed physical picture of such a system. The results of experiments by Klaarenbeek (quoted by Overbeek, 1956,) and Schofield and Talibuddin (1948), which were performed with little or no reference to plant physiology, are also analysed from the D.F.S./W.F.S. point of view; these latter papers provide important quantitative verification of the physical picture to be described.

The ordinary Donnan theory, e.g. equations (1) and (2) above, is a thermodynamic theory, whose basic and unexceptionable postulate is that the electrochemical potential ($\text{const} + RT \ln a + p\bar{V} + Fz\psi$), where a is (chemical) activity, z is the valency of the ion and ψ the potential) of any ion is the same whether the ion is in the external solution or in the Donnan phase. This theory if properly applied will, of course, correctly describe the Donnan system but, like all thermodynamic theories, it gives no insight into what is happening at the molecular level. At this level the "accumulation" of counter-ions and the exclusion of co-ions is simply due to attraction and repulsion by the fixed charge of the Donnan phase. If the fixed charges are not too far apart (not more than about $40 - 50 \text{ \AA}$) then the surface of the polysaccharide micelle can be treated as a uniformly charged surface, which will give rise to an electric double layer in the solution adjacent to the surface. If the fixed charges are negative, and we will henceforth assume this, then mobile cations (counter-ions) are concentrated in the double layer while mobile anions (co-ions) are excluded. This region of the electric double layer is the region of the D.F.S.; any part of the cell wall, water, say, which is not under the influence of an electric double layer will be in the W.F.S. . This latter, qualitative, statement can be made quite quantitative on the basis of the Gouy - Chapman theory of the electric double layer.

Since I merely wish to present a clear physical picture of the situation, it will be assumed that the external solution ions are both monovalent. (A more general case becomes somewhat mathematically cumbersome, though not insoluble.) It will also be assumed that the negatively charged surface can be treated as an infinite plane surface; this assumption, which is not likely to be strictly true, is valid if the "thickness" of the electric double layer is much less than the radius of curvature of the surface bearing the fixed charges. First consider the simplest possible case of a negatively charged (σ coulombs/cm.²) plane surface at $x=0$, with the solution of a uni-univalent salt stretching from $x = 0$ to $x = \infty$, the concentration being C_0 at $x = \infty$. If ψ is the potential at any point x in the solution, ρ the charge density and ϵ the dielectric constant of the medium, then ψ is given by Poisson's equation:

$$\frac{d^2\psi}{dx^2} = - \frac{4\pi\rho}{\epsilon} \quad (5)$$

Now $\rho = (C_+ - C_-)F$ (6)

where C_+ and C_- are the cation and anion concentrations in eq./cc. and F is the faraday. C_+ and C_- are given by Boltzman's equation;

$$C_+ = C_0 \exp(-F\psi/RT) \quad (7a)$$

$$C_- = C_0 \exp(F\psi/RT) \quad (7b)$$

Substituting (6) and (7) in (5) we have:

$$\frac{d^2\psi}{dx^2} = - \frac{4\pi FC_0}{\epsilon} \left[\exp(-F\psi/RT) - \exp(F\psi/RT) \right] \quad (8)$$

The first integration of this gives:

$$\frac{d\psi}{dx} = - \sqrt{\frac{8\pi C_0 RT}{\epsilon}} \left[\exp \frac{F\psi}{2RT} - \exp \frac{-F\psi}{2RT} \right] \quad (9)$$

Now $\left(-\frac{d\psi}{dx} \right)_{x=0} = \frac{4\pi\sigma}{\epsilon}$ (10)

Therefore $\sigma = \frac{\epsilon}{4\pi} \sqrt{\frac{8\pi C_0 RT}{\epsilon}} \left[\exp \frac{F\psi_0}{2RT} - \exp \frac{-F\psi_0}{2RT} \right]$ (11)

Equation (11) thus connects the surface density of fixed charge with the potential, ψ_0 , at the surface. A second integration of equation (9), leads to:

$$x \sqrt{\frac{8\pi F^2 C_0}{\epsilon RT}} = \ln \left[\frac{\left(\exp \frac{F\psi}{2RT} + 1 \right)}{\left(\exp \frac{F\psi}{2RT} - 1 \right)} \frac{\left(\exp \frac{F\psi_0}{2RT} - 1 \right)}{\left(\exp \frac{F\psi_0}{2RT} + 1 \right)} \right] \quad (12)$$

Call $\frac{8\pi F^2}{\epsilon RT} C_0$, which equals $1.06 \times 10^{18} C_0$ cm./eq. for water at 25°C, K^2 .

If $\psi_0 \ll \frac{RT}{F} \approx 25\text{mV.}$, then equation (12) simplifies to:

$$\psi = \psi_0 e^{-Kx}, \quad \psi_0 \ll RT/F \quad (13)$$

and $\frac{1}{K}$ can be called the "width" of the electrical double layer. It is useful even in the more general case of equation (12) to retain this expression for $1/K$. The value of $1/K$ for uni-univalent salt solutions is $0.097/\sqrt{C_0}$ Å if C_0 is expressed in eq./cc, or $3.07/\sqrt{C_0}$ Å if C_0 is expressed in eq./l. Thus at a concentration of 10 meq./l. the "width" of the double layer would be 30.7Å . The approximate equation (13) is rarely adequate in practice except in highly concentrated salt solutions.

In the system so far considered there is no sharp break into a D.F.S. and a W.F.S.; $C_+ > C_-$ all the way from $x = 0$ to $x = \infty$.

However one can work out what the equivalent D.F.S. is. Formally one

can say that the system is equivalent to a D.F.S. stretching from $x = 0$ to $x = x_D$ (to be determined) and a W.F.S. stretching from $x = x_D$ to $x = \infty$, as far as the average concentrations of anions and cations

are concerned. The surface charge has been taken as σ coulombs/cm²;

it is more convenient to express it as Γ equivalents/cm²; thus $\Gamma = -\sigma/F$,

since we are considering that the fixed charge is negative. The concentration

of fixed anion in the D.F.S. is thus $\frac{\Gamma}{x_D}$ eq./cm³ and the mobile cation (c_+)

and anion (c_-) concentrations in this region would be given by the following

equations:

This formula, and theory, of course applies to a single charged

surface in an infinite volume $c_o^2 = c_+ \cdot c_-$. The presence of any other charged

surface causes mutual disturbance of the two double layers and the problem

becomes, mathematically, very difficult. However it can be shown (15)

above theory applies quite well provided the charged surfaces are further

apart than four times the width of the double layer. Therefore $c_o^2 = c_+ (c_- + \Gamma/x_D)$ (16)

neighbouring charged surfaces on the D.F.S. of a cell wall will be qualitatively

Outside the D.F.S. we have W.F.S., i.e. the concentration of both ions is c_o .

So the amount of anion excluded according to this picture is $(c_o - c_-) x_D$.

of the double layer is sufficiently great for the above theory to apply.

If this picture is to be equivalent to the effect produced by the double layer then

$$(c_o - c_-) x_D = \int_0^{\infty} (c_o - c_-) dx \quad (17)$$

where c_- is given by the Boltzman equation (7b) with ψ given

by equation (12). The right hand side of equation (17) is then not too

difficult to integrate and leads to:

It would only occupy 5% of the water volume at 1000mM, and the W.F.S. would

increase from 50% to 55%. The concentration of fixed anion in the D.F.S.

$$(C_0 - C'_-)x_D = C_0 \sqrt{\frac{\epsilon RT}{2\pi c F^2}} \left[1 - \exp \frac{F\psi_0}{2RT} \right] \quad (18)$$

Finally we have from equation (11):

$$- \Gamma F = \sigma = \sqrt{\frac{\epsilon C_0 RT}{2\pi}} \left[\exp \frac{F\psi_0}{2RT} - \exp \frac{-F\psi_0}{2RT} \right] \quad (19)$$

Eliminating ψ_0 and C'_- between equations (16), (18), and (19) leads to the simple result:

$$x_D = 2 \sqrt{\frac{\epsilon RT}{8\pi F^2 C_0}} = \frac{2}{K} \quad (20)$$

Thus the width of the equivalent D.F.S. is exactly twice the "width" of the electric double layer,

This formula, and theory, of course applies to a single charged surface in an infinite volume of solution. The presence of any other charged surface causes mutual disturbance of the two double layers and the problem becomes, mathematically, very difficult. However it can be shown that the above theory applies quite well provided the charged surfaces are further apart than about four times the width of the double layers. The effect of neighbouring charged surfaces on the D.F.S. of a cell wall will be qualitatively considered later on in this article. For the time being it will be tentatively assumed that the ratio of the distance apart of the charged surfaces to the width of the double layer is sufficiently great for the above theory to apply.

It is clear from equation (20) that the width of the double layer and hence the extent (i.e. volume) of the D.F.S. depends on the external concentration of the solutes. Thus in changing from a concentration of 10mM/l to 1000mM/l of a univalent salt, the width of the double layer changes from 30.7Å to 3.07Å and, correspondingly, the volume of the D.F.S. decreases; if the D.F.S. occupied 50% of the water volume when the concentration was 10mM, it would only occupy 5% of the water volume at 1000mM, and the W.F.S. would increase from 50% to 95%. The concentration of fixed anion in the D.F.S.

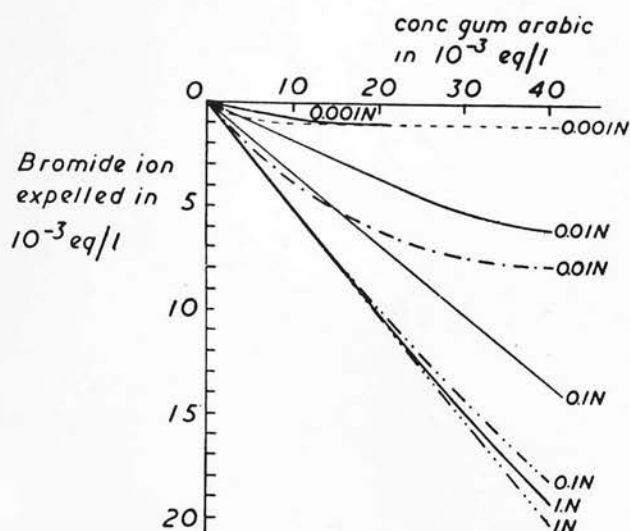


Fig. 5. Expulsion of Br^- ions in the Donnan equilibrium between gum arabic and KBr for four different concentrations of the outside solution. The experimental curves are drawn. The dotted curves represent ideal behaviour.

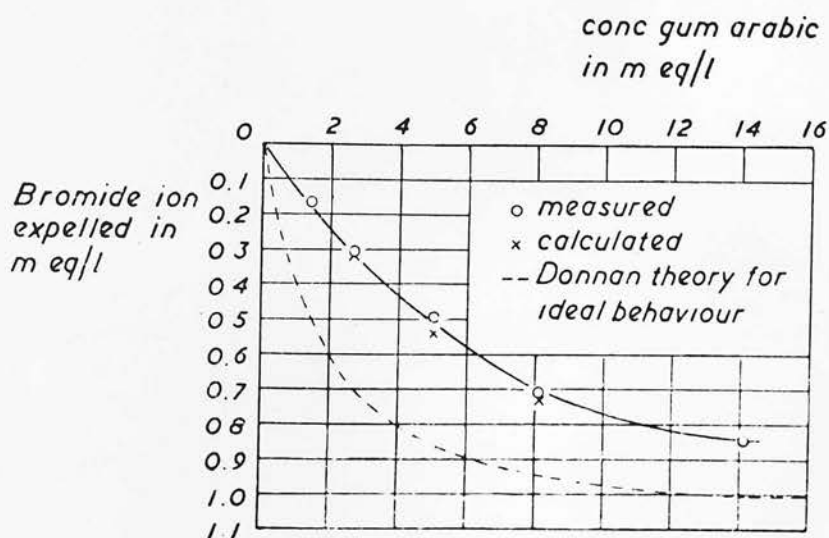


Fig. 6. Expulsion of Br^- ions from solutions of gum arabic with 0.00108 N KBr, as measured (\circ), calculated from eq. (45) (\times), and calculated for ideal behaviour (dotted line).

would also increase by a factor 10. Therefore, in principle, one cannot speak of a D.F.S. and a W.F.S. as if they represented constant volumes of a tissue; their relative proportions and the fixed anion concentration depend on the concentration of the external solution. They also depend on the valency of the ions, the width of the double layer being less the higher the valency. Plant physiologists, so far as I know, have not as yet analysed D.F.S. and W.F.S. measurements with this in mind, although the application of this theory might give valuable information about the cell wall. However there do exist in the literature at least two sets of data which illustrate the above point.

Overbeek (1956) quotes some experimental results obtained by Klaarenbeek on the behaviour, as a Donnan system, of a colloidal solution of gum arabic, separated by a coarse membrane (semi-permeable) from a solution of KBr.

These results are reproduced in Figs. 1 and 2 and in Table 1.

TABLE. 1.

Conc. of KBr in g eq./ per litre	g.eq. Br expelled per g.eq. gum arabic
0.00108	0.115
0.01	0.19
0.1	0.32
1.0	0.50

It is immediately clear from the figures that less Br is expelled from, i.e.

there is a greater concentration of Br in, the gum arabic solution than is expected from a uniform Donnan system; in our phraseology the gum arabic solution comprises both a D.F.S. and a W.F.S. It can be shown quite simply, without making any assumptions about non-overlapping double layers, etc, - indeed without bringing double layers into the calculation - that the relation between V_D , the volume of the D.F.S. per litre of the solution, and q , the amount of Br

expelled in eq./litre, is:

$$V_D = \frac{q}{c_o} \cdot \frac{E - q}{E - 2q}, \quad (21)$$

where c_o is the external KBr concentration in eq./l. and E is the concentration of gum arabic in eq./l. Table 2 gives the results of calculations of V_D from the graph of Fig. 2 together with figures for the "width" of the D.F.S. layer around each gum arabic molecule (M.W. \approx 220,000 , "valency" of molecule \approx 185) and the average distance apart of the molecules.

TABLE 2.

c_o (meq./l.)	E (meq./l.)	V_D (l.)	$2/\kappa$ (Å)	av. sepn. (Å)
1.08	1	0.14	187	677
1.08	2	0.23	187	537
1.08	4	0.44	187	426
1.08	6	0.59	187	373
1.08	8	0.70	187	338
1.08	14	1.00	187	281

It is clear from this table that there is a rough proportionality between volume of the D.F.S. and concentration of gum arabic molecules up to about 6 meq./l. gum arabic concentration, when the average distance apart of the molecules is about four times the thickness of the double layer.

Thereafter the D.F.S. does not increase proportionally and when the separation of the molecules is about three times (3×93) the width of the double layer, the D.F.S. occupies all the solution. Of course this simple-minded approach is vitiated to some extent by the Brownian movement of the molecules, but the mutual repulsion of the negatively-charged molecules tends to counteract this thermal motion and give a lattice-like structure to the solution.

The data given in Table 3 illustrate the variation of the D.F.S. with the concentration of the external solution. The following Table 3 is calculated from Table 1.

TABLE. 3. (Total volume of solution equals 1000 cm.³)

C_o (meq./l.)	$E \left(\begin{array}{c} \text{assume} \\ 1 \text{ meq./l.} \end{array} \right)$	V_D (cm ³)	$2/\kappa$ (Å)	av. sepn. (Å)
1.08	1	115	187	677
10	1	25	61	677
100	1	6	19.4	677

The volume of the D.F.S. markedly decreases as the KBr concentration rises from 1.08 to 100 meq./l., as it should because of the contraction of the double layer. The D.F.S. has, in fact, decreased more than it should - cf. 115/6 with 187/19.4 - but this may be due to experimental error which cannot be judged from the data; one would certainly not expect the overlapping of the double layers to interfere much, but theoretical errors will have arisen through treating a sphere of radius 100Å as a plane surface when the double layer has a thickness of up to 100Å.

This analysis of Klaarenbeek's data illustrates several points: that the D.F.S. varies with external concentration in the expected way; that the D.F.S. extends to twice the width of the double layer provided that the charged surfaces are more than four times the width apart; that when the charged surfaces are less than about three times the width of the double layer apart, the whole of the space between them can be treated as D.F.S.; that the theory cannot be expected to apply too well when the width of the double layer is of the same order of magnitude as the radius of curvature of the charged surfaces.

The other set of results, which are of great biological interest, are in a paper, on "negative adsorption" (of chloride ions) by jute fibres, by Schofield and Talibuddin (1948). These authors measure the amount of chloride excluded by a given weight of jute fibre and express their results as the amount of water from which Cl^- is excluded, v , excluded per 100 g. of dry fibre. Fig. 1 is a representation of one set of their results,

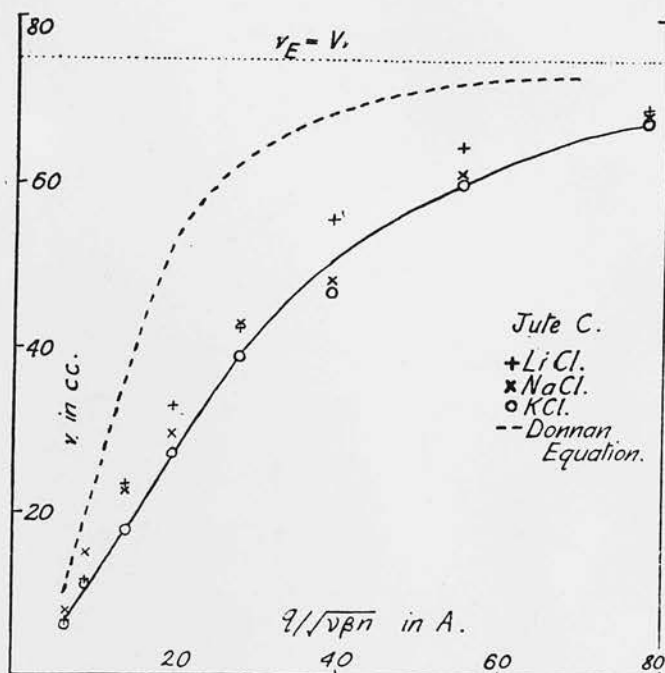


FIG. 1

Fig. 3 . From Schofield and Talibuddin (1948)
 ordinate - volume from which Cl^- excluded
 abscissa - $2/\kappa$ (see text)

The other set of results, which are of direct botanical interest, are in a paper, on "negative adsorption" (of chloride ions) by jute fibres, by Schofield and Talibuddin (1948). These authors measure the amount of chloride excluded by a given weight of jute fibre and express their results as the amount of water from which Cl^- is absolutely excluded per 100 g. of ^{dry} fibre. Fig.3 is a reproduction of one set of their results,

(the abscissa is the same as $2/\kappa$ - the width of the D.F.S., which is inversely proportional to $\sqrt{c_0}$ -); obviously Cl^- is not excluded as much as it would be if the jute were a uniform Donnan system. The volume from which Cl^- is absolutely excluded is not the same as the D.F.S., which is given by

$$V_D = v \cdot \frac{E - v c_0}{E - 2 v c_0} \quad (22)$$

where $v(\text{cc.})$ is the exclusion volume, E is the amount of fixed charge (meq./100 g. dry fibre) and c_0 is the external concentration. From Fig.3 the values given in the following Table 4 have been calculated.

TABLE 4.

V = total water volume = 75cc./100g. dry fibre.

E = 22.0 meq./100g. dry fibre.

C_0 (meq/cc)	V_D (cc.)	$V_D \cdot \sqrt{C_0}$	$2/\kappa (\text{\AA})$	A^- (eq./l.)
1.0	9.15	9.15	6.1	2.40
0.5	14.1	10.0	8.6	1.48
0.2	21.7	9.72	13.7	1.01
0.1	31.0	9.80	19.3	0.71
0.05	42.7	9.55	27.3	0.51
0.025	53.4	8.45	38.6	0.41
0.0125	61.5	6.88	54.6	0.36
0.00625	68.4	5.41	77.2	0.32
(0)	(75)			(0.29)

From this table it can again be seen that the volume of the D.F.S. in this plant material varies markedly with external solute concentration. Between $C_o = 1$ eq./l. and 0.05 eq./l. the volume of the D.F.S. is inversely proportional to $\sqrt{C_o}$, but starts to deviate from this at lower solute concentrations; since the extent of the D.F.S. region is about 27\AA at $C_o = 0.05$ the charged surfaces must be about $50 - 60\text{\AA}$ apart. If the material has a pore geometry, the pores must have diameters of the same order of magnitude. The last column gives the estimated fixed anion concentration in the D.F.S. and this of course also varies as the volume of the D.F.S. varies.

Since at low concentrations the volume of the D.F.S. is approaching the total water volume, it would seem that in jute fibre all the water is within about 100\AA of the fixed negative charges. Thus all the "pores" in jute fibres seem to be charged and have diameters in the range $50 - 100\text{\AA}$. The situation seems to be quite different in the cell wall of Chara australis (Dainty and Hope, 1959,) but it should be noted that "wet" jute fibre contains only 75cc./100g. dry weight whereas "wet" Chara cell wall contains ~~300~~ 300cc./100g. dry weight. The analysis and theory I have presented were not realised or worked out when Dr. Hope and I did the experimental work described in the above paper and thus our results are not extensive enough to admit a detailed discussion on the lines I have used with the papers of Klaarenbeek and Schofield and Talibuddin. However some comments can be made.

The conclusion reached in the paper by Dainty and Hope (1959), that the D.F.S. occupied 7/15 of the total wall water and the W.F.S. occupied 8/15, was based on measurements of the "uptake" of anion (I) from calcium salt solutions of concentrations ranging from 1meq./l. to 20 meq./l.. This uptake was linear with concentration and was approximately a thousand times greater than would have been expected from a uniform Donnan system; it is clear, therefore, that we are dealing with a D.F.S. + W.F.S..

For the same (molar) concentration the double layers are narrower with a solution such as CaCl_2 than with, say, KCl . A calculation shows that at concentrations of CaCl_2 (or CaI_2) of 1meq./l. and 20meq./l. the width of the double layers are 100\AA and 22\AA . Therefore if the fixed charges are confined to pores less than about 70\AA in diameter there should be no variation of the volume of the D.F.S. in the range of the concentrations used. It would seem that this is the most likely situation, i.e. that the fixed charges are confined to "pores" of diameter less than 70\AA and that an approximately equal volume of uncharged pores are present into which both ions of the salt have equal access. The other possibility - that there are some very wide charged pores, of diameter much greater than $3 - 400\text{\AA}$, to accommodate the W.F.S. - seems to me, at the moment, less likely for I would expect this to show up in deviations from linearity in the uptake of the anion. However much more, obvious, experimentation is needed and experiments are being planned on cell walls of Nitella translucens with the above analysis and theory as a guide. In this way it is hoped to make a big step forward in understanding the passive uptake and exchange of ions with the free space of plant tissue.

The experiment to be analysed is the following. The tissue is pretreated to ensure that all the counter-ions in the D.F.S. are the same as the cations to be used in the experiment. The tissue is then equilibrated for some time in the appropriate salt solution of concentration C_0 moles/ml.³ and then transferred to a salt solution of the same concentration and chemical composition but in which one or both of the ions is an isotope. The passive uptake of this isotope by the tissue is then measured as a function of time by suitable experimental techniques.

The other "free space problem" to discuss is to what extent the protoplasm of the cells of a tissue contribute to the free space if the protoplasm is bounded by a plasmalemma; it is logical to preface this with some remarks on the kinetics of passive readjustment (uptake or loss and exchange) of the solutes of the free space. This discussion will be confined to the more interesting, complex, and important case of an ionised salt solution.

Briggs (1957) has discussed the kinetics of passive readjustment and, though he assumes that the D.F.S. is in the protoplasm, much of his theory can be applied wherever the D.F.S. is. However his discussion is rather difficult and curtailed and, in at least one instance, I believe it is not quite correct. Instead of trying to discuss the problem in general, I shall discuss the isotopic exchange of anions and cations between external solution and a disc of storage tissue such as beet. This example is chosen because it avoids the great difficulties of the kinetics of uptake of a salt, when transient diffusion potentials occur, and also because the best experimental work has been done by this technique on beet storage tissue (Briggs, Hope and Pitman, 1957). In my opinion, too, these authors have not quite understood some of the implications of their results, or at least they have given this impression.

The experiment to be analysed is the following. The tissue is pretreated to ensure that all the counter-ions in the D.F.S. are the same as the cations to be used in the experiment. The tissue is then equilibrated for some time in the appropriate salt solution of concentration C_0 moles/cm.³ and then transferred to a salt solution of the same concentration and chemical composition but in which one or both of the ions is an isotope. The passive uptake of this isotope by the tissue is then measured as a function of time by suitable experimental techniques.

A similar problem to this has been much studied by animal physiologists; this is the exchange of ions between external solution and a tissue such as muscle or nerve, which consists of an extracellular space and a large number of small cells. Harris and Burn (1949), Keynes (1954) and Dainty and MacRobbie (unpublished, but applied in analysing the experimental data of Dainty and Krnjević, 1955,) have developed mathematical analyses of the exchange kinetics for these tissues. An attempt will first be made to apply similar methods to the exchange with plant tissues, but it can be said at the outset that these methods, which are satisfactory for animal tissues, fail; however a great deal can be learned from the failure.

The basic assumption to be made, as was done for the animal tissues, is that the exchange is an in series process, i.e. that the isotope taken up by the D.F.S. has diffused through the W.F.S. in order to reach the D.F.S. An additional assumption is made in order to make the problem mathematically tractable; this is that the D.F.S. is distributed at random throughout the W.F.S. in small pieces (so that there is no large difference in concentration of the isotope in the W.F.S. over the length of a piece of D.F.S.). With these assumptions the isotope concentrations in the W.F.S. and the D.F.S. (C_w^* and C_i^*) must satisfy the following differential equation if the tissue is in the form of a large, thin, disc of thickness 2ℓ :

$$\epsilon \frac{\partial C_w^*}{\partial t} + \mu \frac{\partial C_i^*}{\partial t} = \epsilon D' \frac{\partial^2 C_w^*}{\partial x^2} \quad (1)$$

where x is measured perpendicular to the disc, ϵ and μ are the volume fractions of the tissue occupied by W.F.S. and D.F.S. respectively, and

D' is the self-diffusion coefficient of the ion considered in the W.F.S.; D' is less than the self-diffusion coefficient in an ordinary solution

because the ion movement is hindered by obstructions in the tissue

(see ^{Mackie and} Meares, 1955, for the calculation of D'): In order to solve this equation we must have another equation connecting C_w^* and C_i^* .

Two cases will be considered: case I, in which the diffusion coefficient

in the D.F.S. and the size of the D.F.S. pieces are such that the D.F.S. is in "instant" equilibrium with the isotope concentration at its surface, and case II in which the diffusion of the isotope in the D.F.S. is so slow that the exchange between the external solution and the W.F.S. is practically complete before there is any appreciable exchange with the D.F.S. The intermediate case, which can be solved for an animal tissue, is mathematically very difficult for a plant tissue; fortunately a qualitative consideration of the intermediate case is adequate.

In case I the second equation connecting C_w^* and C_i^* is, for dilute solutions, from the Donnan equations,

$$\frac{C_i^*}{C_w^*} = \frac{A}{C_o} \quad \text{for the cation and} = \frac{C_o^2}{A} \quad \text{for the anion} \quad (2)$$

$$\text{i.e.} \quad C_i^* = k C_w^*, \quad \text{where } k = A/C_o \text{ or } C_o^2/A; \quad (3)$$

A is the concentration of the fixed anion in the D.F.S. and C_o is the external salt (uni-univalent) concentration. On substituting

equation (3) in equation (1), we have

$$(\epsilon + \mu k) \frac{\partial C_w^*}{\partial t} = \epsilon D' \frac{\partial^2 C_w^*}{\partial x^2},$$

leading to

$$\frac{\partial C_w^*}{\partial t} = D \frac{\partial^2 C_w^*}{\partial x^2} \quad (4)$$

where $D = \frac{\epsilon D'}{\epsilon + \mu k}$ and $C^* = \epsilon C_w^* + \mu C_i^* = \text{average concentration.}$

Equation (4) is a standard equation, which can easily be solved for C^* ;

C^* can then be integrated over the disc, leading to:

$$1 - \frac{M_t^*}{M_\infty^*} = \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left(-D(2n+1)^2 \pi^2 t / 4l^2\right) \quad (5)$$

Where M_t^* is the total uptake of isotope after t seconds and M_∞^* is the uptake after infinite time. This equation is identical with that quoted by Briggs

(1957). If $\ln(1 - M_t^*/M_\infty^*)$ is plotted against t , there is a small initial fast fall followed by a straight line of slope $D\pi^2/4l^2$.

Hence D can be deduced from the experimental results. According to the assumptions made, $D = \epsilon D' / (\epsilon + \mu k)$; for beet tissue $\epsilon = 0.05$,

$\mu = 0.02$, $k = 550 \times 10^{-6}/C_0$ for a monovalent cation and $C_0/550 \times 10^{-6}$ for a monovalent anion. The range of values of C_0 used by Briggs, Hope and Pitman were 1 to 20×10^{-6} moles/cm³. Therefore for a cation $\mu k \gg \epsilon$

and $D \approx \epsilon D' / \mu k = D' C_0 / 220$; for an anion $\mu k \ll \epsilon$, thus $D \approx D'$. Since $D = D'$ for the anion, the measured value of D for the anion can be used to estimate the value of D for the

cation. Applying this theory to the results of Briggs, Hope and Pitman, there is complete disagreement; they find that the apparent diffusion coefficient of the cation does not change with concentration from 1 to 20 mM./l. and this is quite contrary to the above theory. Also the magnitude of the

cation diffusion coefficient is quite wrong - though this seems to have escaped their notice. They find that D for I^- is 6.9×10^{-6} and for Rb^+ is 1.1×10^{-6} , independent of concentration. The above theory indicates that the maximum value of D for Rb^+ should be about 10^{-6}

at $C_0 = 20$ mM; at $C_0 = 1$ mM it should be twenty times less. Thus the experimental results are quite incompatible with the assumption that the isotopes diffuse through the W.F.S. up to the D.F.S. and then rapidly equilibrate with the D.F.S.

Briggs, Hope and Pitman recognise this and say: "since the concentration in the W.F.S. is 95% of C_0^* within 5 min. - - - the equilibration of the

D.F.S. is probably limited in time by exchange between the W.F.S. and the D.F.S." If this is the correct explanation (case II) then, for the cation, there would be a rapid exchange with the W.F.S. followed by a slow exchange between the W.F.S. and the D.F.S. The experimental curve of $\ln (1 - m_t^*/m_{\infty}^*)$ against time would look much the same as in case I, but the slope of the linear portion of the curve would be given by $D_d \pi^2 / 4 \delta^2$ where D_d is the self-diffusion coefficient of the cation in the D.F.S. and δ is the thickness of the D.F.S. From the experimental results of Briggs, Hope and Pitman, I calculate that if $\delta = 1 \mu$, $D_d = 4 \times 10^{-12} \text{ cm.}^2 \text{ sec.}^{-1}$ for Rb^+ ! This is certainly several orders of magnitude too small and is in complete disagreement with a second experiment they did in which they measured the rate of passage of an isotope across a disc of tissue; to explain their results in this second experiment, they assume $D_d = 1 \times 10^{-5} \text{ cm.}^2 \text{ sec.}^{-1}$. Clearly the basic assumption of this theory (case I or case II or, indeed, an intermediate case) must be wrong.

The fact that the apparent diffusion coefficient of a cation is independent of concentration indicates that exchange with the W.F.S. and exchange with the D.F.S. cannot be in series. Also the D.F.S. is not of course distributed in small pieces at random throughout the tissue; it is distributed in relatively large spherical shells touching each other. (A 1mm. disc of beet tissue is about 10 cells thick.) In order to explain the experimental results we must assume that the uptake of isotope by the W.F.S. and the D.F.S. proceed largely in parallel; i.e. that the isotope is initially taken up in both the W.F.S. and D.F.S. at the surface of the disc and then proceeds to diffuse independently along the two free spaces. Of course there will be some exchange between the W.F.S. and the D.F.S. but this must be relatively small compared with diffusion along the D.F.S., otherwise the apparent diffusion coefficient of the cation would show some concentration dependence.

With the above assumptions the uptake of an isotope of a cation is given by

this discussion of the certain existence of an unstirred layer of external solution, about 100 μ thick, bounding the two external surfaces of the disc. However it can be shown that with such a disc of storage tissue, this unstirred

$$\frac{M_t^*}{C_0^*} = \epsilon \left[1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \cdot \exp \left(-D'_w (2n+1)^2 \pi^2 t / 4l^2 \right) \right] + \mu k \left[1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \cdot \exp \left(-D'_d (2n+1)^2 \pi^2 t / 4l^2 \right) \right] \quad (6)$$

where D'_w , D'_d are the self-diffusion coefficients in the W.F.S. and the D.F.S., respectively, and the other symbols have the same meaning as before. For the cation, since $\mu k \gg \epsilon$ the second term is predominant; for the anion, since $\mu k \ll \epsilon$, the first term only is important.

When the results of Briggs, Hope and Pitman are analysed on the basis of this theory, they fit quite nicely in so far as the theory predicts no concentration dependence of the apparent diffusion coefficient. On this theory the measured diffusion coefficients are actually equal to the diffusion coefficients in the W.F.S. (anions) and the D.F.S. (cations). Thus the correct interpretation of these experimental results would seem to be that they prove that, for a cation, diffusion along the W.F.S. and the D.F.S. take place in parallel and that the diffusion coefficient of I^- in the W.F.S. is 6.9×10^{-6} , of Rb^+ in the D.F.S. is 1.1×10^{-6} and of Sr^{++} in the D.F.S. is 3.7×10^{-7} - all in $cm.^2 sec^{-1}$. This seems to me to be an important conclusion.

The values of the self-diffusion coefficients of Sr^{++} and Rb^{+4} in the D.F.S. seem to be of the right order of magnitude as judged by measurements on cation diffusion coefficients in ion-exchange resins. The value of $6.9 \times 10^{-6} cm.^2 sec^{-1}$ for I^- is not much smaller than the value in free solution at $20^\circ C$, indicating that the W.F.S. is not ^a highly-tortuous channel system, but can be better approximated as a series of tubes - with bulges - going through from one face of the disc to the other.

It will probably have been noticed that no account has been taken in this discussion of the certain existence of an unstirred layer of external solution, about 100μ thick, bounding the two external surfaces of the disc. However it can be shown that with such a disc of storage tissue, this unstirred

layer has a negligible influence on the exchange kinetics. This is essentially a question of relative dimensions of the disc and the unstirred layer; with the thin (16μ) cell wall of Chara australis, the unstirred layer entirely controls the cation exchange kinetics (Dainty and Hope, 1959).

The question as to whether the protoplasm contributes anything to the D.F.S. of a tissue is a very difficult one. Of course, if there is no plasmalemma then there is no problem; it is all part of the D.F.S. However if the protoplasm of the vacuolated cells of higher plants is at all similar to that of Nitellopsis obtusa or to that of animal cells, then some plausible guesses can be made about its contribution to the D.F.S. of, say, beet storage tissue. Thus the flux of the ions sodium, potassium and chloride across the plasmalemma should be of the order of 10^{-12} moles/cm.²/sec. at 2°C and the fluxes of such ions as calcium, magnesium and sulphate should be much less, in fact the D.F.S. as measured by the exchange of a divalent ion should all be in the cell wall.

From the results of Briggs, Hope and Pitman (1957), the monovalent cations in the D.F.S. of beet tissue exchange with those in the external solution at a rate which has a time constant of about 1000 secs. If the specific activity in the cell wall were equal to the external specific activity then in 1000 secs about 10^{-9} moles/cm.² would exchange across the plasmalemma; for a cell of diameter 100μ , this would be an exchange of about 3×10^{-13} moles. If the cation involved were K, its concentration in the protoplasm might be expected to be about 300-400mM. and, therefore, if the protoplasm is 1μ thick, the amount of K in the protoplasm would be about 10^{-11} moles. So in 1000 seconds only about 3% of the internal potassium would exchange, even if the cation in the cell wall had reached saturation specificity. A more detailed calculation in fact suggests that - with the above figures - only a small percentage of the D.F.S. of beet

storage tissue could be due to the protoplasm. The fraction is likely to be much less than this for any other ion, because, if the protoplasm is anything like Nitellopsis or animal protoplasm, K is the most abundant ion. This conclusion should not, of course, be accepted as final but should be looked upon as a guide to future experiments to determine the ionic properties of the protoplasm of higher plant cells.

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Table 1

Depth cm.	Exposure hr.	Concentration mg./g.	Exposure hr.	Concentration mg./g.
0	0	0	0	0
1	1	1	1	1
2	2	2	2	2
3	3	3	3	3
4	4	4	4	4
5	5	5	5	5
6	6	6	6	6
7	7	7	7	7
8	8	8	8	8
9	9	9	9	9
10	10	10	10	10

STUDIES ON THE UPTAKE OF PHOSPHORUS FROM ^{32}P -LABELLED SUPERPHOSPHATE BY CROPS. I. OATS.

The curves showing the rate of increase in sorption and pressure. It is clear that but it is to be noted that in a shallow layer an equilibrium has been established between methyl bromide and the soil. This has been why the increase during the exposure to rise at a rapid rate. The instantaneous action demonstrated by wheat feed at 5 cm. depth.

By contrast with wheat feed at 5 cm. depth, since in the case of oats, change might be effected by wheat feed at 5 cm. depth. A thermometer was used to the temperature noted, avoided quickly, the expansion is approximately

STUDIES ON THE UPTAKE OF PHOSPHORUS FROM ^{32}P -LABELLED SUPERPHOSPHATE BY CROPS. II. POTATOES.

In the case of bromide, the full atmospheric pressure at the reduced pressure was correspondingly period at atmospheric pressure. This may be explained immediately in contact with the soil. From the small-scale tests, the reduced pressure, the higher due to more rapid penetration of

Conclusions

To sum up the results the wheat feed appears to be absorbed by wheat feed appears to be absorbed at the surface is not clear. With wheat feed, presumably as a result of the rapid penetration of

of these products, however, is the direct effect of the reduction of pressure on the sorption of methyl bromide a large one nor should the direct effect on the size of the residual bromide be important.

In the practical fumigation of these commodities in commercial packages, the increased rate of penetration into the package obtained in a treatment at reduced pressure may well result in an over-all increase in the proportion of the dose of fumigant which is absorbed in a given period, but this loss may be more than offset by the reduction in dose made possible by the improved distribution of fumigant through the package. It should also follow that the lower dose will result in a lower mean level of residual bromide throughout the package.

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STUDIES ON THE UPTAKE OF PHOSPHORUS FROM ^{32}P -LABELLED SUPERPHOSPHATE BY CROPS. I.—Oats

By R. D. VERMA, J. DAINTY and K. SIMPSON

Soils from two sites, one high and one low in available phosphorus, were used in a greenhouse pot experiment with different rates of application of ^{32}P -labelled superphosphate in combination with various dressings of ammonium sulphate. The crop was sampled at three stages of growth.

The pattern of uptake was different in the two soils. In the low-phosphate soil the percentage of fertiliser-derived phosphorus in the plant fell steadily as the season progressed, but in the high-phosphate soil there was apparently a renewed demand for phosphorus by the plant at the time of grain formation. This demand was met by fertiliser-phosphorus. There was no indication that the extra uptake during this period enhanced grain formation. Grain yield was, in fact, reduced by the application of 160 lb. of P_2O_5 per acre as superphosphate.

The uptake of soil-phosphorus was depressed so much by applications of superphosphate that the total uptake in treated pots from both soils was less than that in the control at maturity.

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Introduction

There has been a large increase in the use of phosphate fertilisers in recent years, amounting in Great Britain to more than 100% between 1939 and 1953. This is accounted for by an increase in the acreage under cultivation, in the percentage of phosphorus in fertilisers and in rates of application per acre.

The experiment reported here is one of a series being carried out to investigate the possibility of economies in the rate of application of phosphate fertilisers to various crops. In three earlier reports published by Simpson,¹⁻³ chief emphasis was laid on the effects of phosphatic fertiliser on the final crop yield. Subsequent work has been concerned with the influence of phosphates, in association with nitrogenous and potassic fertilisers, on the development and composition of the crop.

In this experiment, ^{32}P -labelled superphosphate was used with the object of examining, on soils high and low in 'available' phosphorus, the following points: (1) the effect of different applications of superphosphate and ammonium sulphate in combination on the yield of shoot and grain (oats) at different stages of growth, (2) the relative extents of uptake of fertiliser- and soil-phosphorus by different parts of the plant during the season and (3) the efficiency of the applied phosphorus in increasing crop yield and the phosphorus content.

Experimental

Preparation of ^{32}P -labelled superphosphate

Early in 1955, ^{32}P -labelled superphosphate was prepared from pile-irradiated red phosphorus by the Radiochemical Centre, Amersham, Bucks. The process consisted in burning 15 g. of radioactive red phosphorus to P_2O_5 and treating this with the minimum amount of sulphuric acid so that the resultant solution contained orthophosphoric acid in a solution which had at least 70% by weight of sulphuric acid. The mixture was held at 100° for 2 hours to ensure that all phosphorus was present as orthophosphate. Then 333 g. of this mixed acid (322 g. of 75% H_2SO_4 and 11.3 g. of 100% H_3PO_4) were added to 400 g. of ground phosphate rock, mixed thoroughly for 2 min. and then heated in an oven at 95° for 2-3 hours. The resultant product was in excellent physical condition, closely resembling commercial superphosphate, and contained 18.8% of water-soluble P_2O_5 .

Soils

Two soils were used, known to contain quite different amounts of easily soluble phosphorus. The first was a loam derived from an 18-in. layer of water-worked till, pH of aqueous extract 6.2, and 'available' P_2O_5 260 p.p.m. The other soil was slightly heavier in texture but of similar parent material, and had pH 5.9, 'available' P_2O_5 25 p.p.m. The two soils are referred to throughout this paper as 'high-P' and 'low-P' soils. The method used for estimation of available phosphorus was that described by Kirsanov⁴ using 0.2N-HCl as extractant. Estimations of available phosphorus in the two soils were also made on extracts prepared with citric acid, acetic acid and with ammonium acetate-acetic acid buffer (pH 4.5). The results obtained agreed with those determined by the Kirsanov method.

After air drying and sieving (0.5-in. mesh), the soil was mixed with an equal weight of nutrient-free coarse sand (2 mm.), and one-third of the mixture was placed in 10-lb. pots at the rate of $3\frac{1}{3}$ lb. per pot. The remainder was mixed with the fertilisers before being added to the pots.

Six kg. of ^{32}P -labelled superphosphate (approx. 100 $\mu\text{C/g}$. superphosphate) was handled in this and associated experiments on potatoes reported elsewhere.⁵ The requisite amounts of superphosphate were weighed out and added to the corresponding amounts of nitrogen and potassium fertilisers in 8-oz. bottles. Protective clothing, face masks and rubber gloves were worn during the handling of this material both in the laboratory and in the field. Careful monitoring showed no contamination at any stage of the experiment.

Treatments and layout

Superphosphate at four rates—equivalent to 0, 40, 80 and 160 lb. P_2O_5 per acre (equivalent approximately to 0, 3.6, 7.2 and 14.4 μC of ^{32}P per pot)—was used in all possible combinations

with ammonium sulphate at three rates—0, 40 and 80 lb. of nitrogen per acre. The twelve treatment combinations were replicated five times in randomised blocks, for both soils, giving a total of 120 pots. Potassium chloride at a rate equivalent to 60 lb. of K_2O per acre was added to all pots. Fertiliser treatments were calculated on the basis of 2×10^6 lb. of soil per acre.

Sowing and sampling

Forty oat seeds (variety Blenda) were sown in each pot at a depth of $\frac{3}{4}$ in. and the pots watered to field capacity on alternate days. Emergence began after seven days. Germination was uniform and was complete 11 days after sowing. The plants were then thinned out to 30 per pot.

The first samples of 10 plants per pot were taken, 31 days after sowing, when the plants were about 6 in. high, the plants being removed by cutting the stem just above soil level. Harvested plants were immediately weighed in the glasshouse and their dry weight subsequently determined in the laboratory. A second sample of 10 plants was taken 54 days after sowing (just before the emergence of the ear) and the remaining 10 plants were allowed to mature, being harvested 94 days after sowing. Fresh and dry weights of both shoot and grain were recorded for the last samples. At all samplings the five replicate dried samples were combined, milled and analysed for total P and ^{32}P .

Analytical methods

Total phosphorus determination.—The method described by Piper⁶ was used, the digest being filtered and collected in a 100-ml. graduated flask from which aliquot samples were taken for total phosphorus determination. Because of the decay in radioactivity, 8-g. samples of dry plant material were used at the third sampling instead of the usual 2-g. In this case the amount of digesting acid was doubled.

Measurement of ^{32}P .—Ten-ml. aliquots from the above solution were counted in an M6 liquid counter using a standard scaling unit and power pack. The usual corrections were made for background counts and dead time. The counting rate at any time was compared with that of a standard solution prepared from the original superphosphate; this procedure essentially corrected for the radioactive decay. From the standardised counting rate the amount of phosphorus derived from fertiliser was calculated.

Results

The weight of oat shoots (dry matter) per 100 plants is shown in Table I for all samplings for both high- and low-P soils. The percentage of dry matter in the plants was only slightly affected by treatments.

In both high- and low-P soils, nitrogen dressings were relatively ineffective at the first sampling, but showed marked effects later in the season. The higher level of nitrogen (80 lb. per acre) was significantly better than the 40 lb. per acre level on both soils at the second sampling and only on the high-P soil at the third sampling.

After the first sampling, where added phosphorus was effective only at the first level (40 lb./acre), there was no response to phosphate applications, except on the low-P soil at maturity. There was no significant difference between the yields produced by added phosphorus at different rates and there was no interaction between nitrogen and phosphorus treatments.

The percentage of P_2O_5 (total) in the dry matter of the shoot samples at three stages of growth is shown in Table II. This table shows that the P_2O_5 content of oat shoots (dry matter) grown in the high-P soil is, at all stages and under all treatments, from 25 to 100% greater than that of plants from the low-P soil. Both applications of ammonium sulphate on both soils decreased the percentage of P_2O_5 as did the dressing of 40 lb. per acre of P_2O_5 as superphosphate.

Among treatments on both soils, increases in the rate of application of superphosphate increased the percentage of P_2O_5 in the shoot. As the season progressed, the P_2O_5 content of the shoot decreased, the fall being very marked between the second and third observations—the period of grain formation.

The percentage of the absorbed phosphorus which was derived from fertiliser is shown for both soils in Fig. 1, from which it is obvious that the plants from the low-P soil derived

Table I

Yield of shoot (dry matter) in g. per 100 plants at three stages of growth and with different fertiliser treatments

Number of days after sowing	P_2O_5 applied, lb./acre				L.S.D. \pm	
	0	40	80	160	$P = 0.05$	$P = 0.01$
Low-P soil						
31	9.6	11.2	12.0	13.5	0.9	1.2
54	29.4	29.9	32.0	36.5	N.S.	N.S.
94	45.0	48.8	51.2	51.1	3.5	4.7
High-P soil						
31	11.4	11.7	11.9	13.5	0.31	0.42
54	34.7	38.8	37.5	39.2	N.S.	N.S.
94	75.3	74.4	73.3	75.8	N.S.	N.S.
Number of days after sowing	N applied, lb./acre				L.S.D. \pm	
	0	40	80		$P = 0.05$	$P = 0.01$
Low-P soil						
31		11.4	11.3	12.1	N.S.	N.S.
54		27.9	32.9	34.4	1.5	2.0
94		41.8	49.7	53.4	5.8	7.8
High-P soil						
31		11.6	12.1	12.7	N.S.	N.S.
54		35.1	38.9	40.3	2.6	3.4
94		64.1	76.7	90.6	8.5	11.4

L.S.D. = Lowest significant difference

N.S. = Not significant

Table II

Percentage of P_2O_5 in the dry matter of shoot samples at different stages of growth as affected by fertiliser treatments

Number of days after sowing	P_2O_5 applied, lb./acre				N applied, lb./acre		
	0	40	80	160	0	40	80
Low-P soil							
31	0.46	0.42	0.47	0.59	0.52	0.50	0.46
54	0.45	0.39	0.39	0.42	0.43	0.40	0.41
94	0.15	0.12	0.14	0.15	0.19	0.12	0.11
High-P soil							
31	0.70	0.67	0.73	0.89	0.81	0.71	0.73
54	0.52	0.49	0.51	0.63	0.63	0.51	0.48
94	0.24	0.22	0.25	0.38	0.45	0.23	0.15

a much higher percentage of their total uptake from the fertiliser than those from the high-P soil. In the low-P soil there was a steady fall throughout the season in the percentage of phosphorus derived from fertiliser. In the high-P soil, however, the percentage of this phosphorus fell sharply between the first and second observations and rose again at maturity. Irrespective of the amount of available soil phosphorus, each increase in the rate of phosphate application led to an increase in the percentage of phosphorus derived from the fertiliser. This increase was almost linear with rate of application in the low-P soil, but in the high-P soil it fell away rapidly above the 80 lb. P_2O_5 per acre level.

Data showing the effect of treatments on the yield of grain and uptake of phosphorus by the grain are given in Table III.

It will be seen from Table III that the fresh weight of grain was adversely affected by phosphate applications. On both high- and low-P soils significant depressions in grain yield were produced by the dressing with 160 lb. of P_2O_5 per acre and on the deficient soil application of 80 lb. per acre also significantly reduced grain yield. Nitrogen applied at 40 lb. per acre gave rise to very good increases in grain yield and a further highly significant increase was recorded on the high-P soil at a nitrogen level of 80 lb. per acre. As in the yields of shoots, there was no significant interaction between N and P treatments.

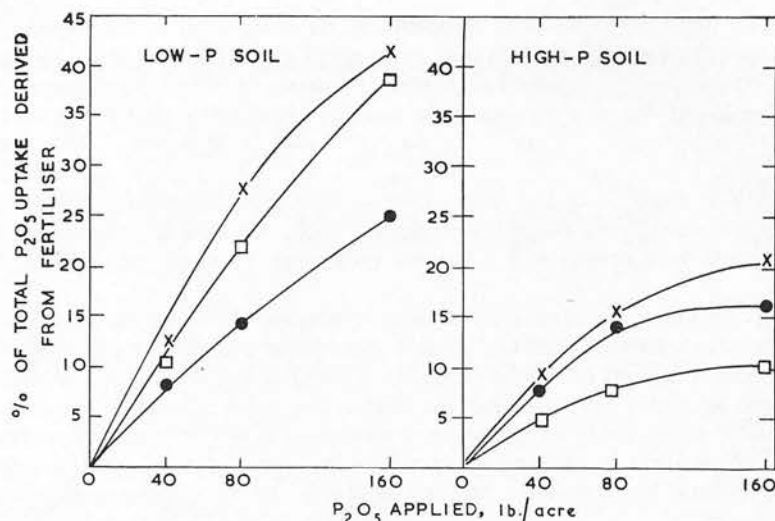


FIG. 1.—Percentage of total P_2O_5 uptake derived from fertiliser
 × 1st sampling □ 2nd sampling ● 3rd sampling

The percentages of dry matter and of total P_2O_5 in the grain were remarkably constant both between soils and between treatments. The percentage of fertiliser-derived P_2O_5 in the grain followed a similar pattern to that in the shoots, increasing linearly with fertiliser application in the low-P soil but falling away at higher levels of application in the high-P soil.

Table III

Yield and uptake by grain of total and fertiliser-phosphorus as affected by fertiliser treatments

	P ₂ O ₅ applied, lb./acre				L.S.D. ± P=0.05	N applied, lb./acre			L.S.D. ± P=0.05
	0	40	80	160		0	40	80	
Low-P soil									
Fresh weight, g./100 plants	231	235	211	212	17.1	192	239	236	22.5
Dry matter, %	89.0	89.1	89.6	89.4	N.S.	89.4	89.1	89.3	N.S.
P ₂ O ₅ in dry matter, %	1.10	1.06	1.12	1.13		1.13	1.09	1.09	
% of total P ₂ O ₅ derived from fertiliser	—	7.4	14.3	28.3		16.5	16.9	16.4	
High-P soil									
Fresh weight, g./100 plants	227	214	213	191	14.7	169	202	269	21.1
Dry matter, %	87.9	88.0	88.2	88.6	N.S.	88.0	88.1	88.4	N.S.
P ₂ O ₅ in dry matter, %	1.19	1.18	1.16	1.22		1.22	1.19	1.16	
% of total P ₂ O ₅ derived from fertiliser	—	7.1	14.0	15.2		10.3	13.0	12.9	

Fig. 2 shows the uptake of total and fertiliser-phosphorus in g. per 1000 plants under different phosphate treatments at all samplings. (Different scales are used for the ordinates of the two graphs.) The curves for fertiliser-phosphorus uptake show linearity on the low-P soil and curvature on the high-P soil. The uptake of soil-phosphorus from the deficient soil was progressively depressed, as the season advanced, by increased dressings of applied superphosphate. This effect was so marked that, at maturity, the highest uptake of total phosphorus was from the control pots. This effect was reflected in the yields of grain. A different picture obtains in the high-P soil. While the lower rates (40 and 80 lb. of P_2O_5 per acre) of superphosphate depressed uptake of soil-phosphorus, the highest rate (160 lb.) stimulated it so much that the total uptake was greater than that of the control plants. This effect was not reflected in increased, but in decreased yields of grain.

Discussion

Applications of both superphosphate and ammonium sulphate increased the amount of vegetative growth of oats at some time during the season. The beneficial effect of readily

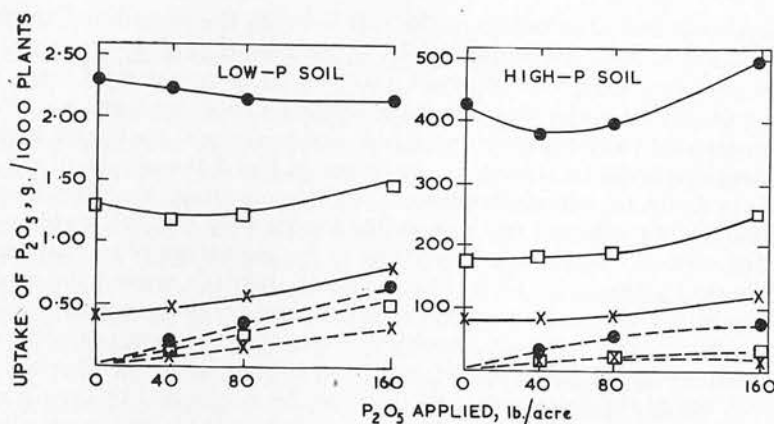


FIG. 2.—Total and fertiliser P_2O_5 in g./1000 plants

× 1st sampling
□ 2nd sampling
● 3rd sampling

— total P_2O_5
- - - fertiliser P_2O_5

available phosphorus was noticeable only in the early part of the season (Table I). The differences in the growth of plants due to added phosphorus were visible soon after germination and two weeks after emergence it was possible to distinguish visually the plants on soils treated with phosphate. As is also shown in Table I the visual effect had disappeared by the second sampling (54 days after sowing) and there was only a slight effect on the deficient soil at maturity.

The effect of the nitrogenous fertiliser followed a different pattern, no stimulation of growth being noticeable at 31 days. The effect at 54 days, shown in Table I, was visually obvious and remained so until the plants were mature. The delayed action of the ammonium sulphate may be the result of a reduction of the micro-organism population by air-drying of the soils before the experiment was started. If so, apparently a period of more than 3 weeks was necessary for the re-establishment of the nitrification processes. This lack of available nitrogen from the applied fertiliser during the early stages of growth may also explain the absence of any stimulation of phosphorus uptake by applied nitrogen until the period of grain formation. Contrary to the findings of Coleman,⁷ Dumenil & Nelson⁸ and Bennett *et al.*,⁹ applications of nitrogen did not increase the phosphorus content of the plants.

Despite the early growth stimulation, the final yields of grain (Table III) were depressed on both soils by large dressings of fertiliser phosphorus. There was thus no indication that even on the low-P soils, any phosphate dressing would have been beneficial under the experimental conditions. Soil temperatures in this experiment were naturally higher than those under field conditions and this may have produced some increase in availability of soil-phosphorus. This could not be detected, however, by soil analysis at the end of the season.

The yields of grain on the two soils (Table III) were very similar and it appears that even the low-P soil was able to supply sufficient phosphorus for optimum grain production. From Fig. 2 it will be seen that the high-P soil supplied almost twice as much soil-phosphorus as the low-P soil, but this extra phosphorus did not give any higher yield of grain.

The grain yield was vigorously stimulated by fertiliser-nitrogen, particularly in the high-P soil.

The depressions in the percentage of total phosphorus in the dry matter (Table II) effected by superphosphate levels of 40 and 80 lb. of P_2O_5 per acre are difficult to explain. As may be deduced from Fig. 2, they appear to arise from large depressions in the uptake of soil-phosphorus accompanying smaller increases in the uptake of fertiliser-phosphorus.

Nitrogen applications depressed the percentage of phosphorus in the shoot, particularly at the sampling of the mature plants. This depression is associated with increased vegetative growth and also with increases in grain weight under the influence of the nitrogenous fertiliser.

Fig. 1 shows the pattern of the percentage of uptake of total phosphorus derived from fertiliser. In the low-P soil as much as 40% at the first sampling was derived from fertiliser compared with a maximum of 20% in the high-P soil. The percentage of fertiliser-derived phosphorus fell steadily throughout the season in the low-P soil and Fig. 2 shows that practically

no fertiliser-phosphorus was absorbed from this soil between the second and third samplings. One explanation of the decrease in specific activity of the crop during growth would be isotopic exchange. There is some disagreement about this process as a source of error in assessing fertiliser-phosphorus uptake. The subject is thoroughly reviewed by Mattingley,¹⁰ who states that, if the estimate of fertiliser-phosphorus is low due to isotopic exchange, high values for uptake of soil-phosphorus will be obtained. As shown in Fig. 2 the uptake of soil-phosphorus is depressed substantially by added phosphorus. It is more likely therefore that phosphate fixation in this moderately acid soil was responsible for the very low uptake of fertiliser-phosphorus later in the season. In the high-P soil (Fig. 1) the percentage of fertiliser-derived phosphorus fell rapidly during the period of vegetative growth (between 1st and 2nd samplings), but there appeared to be a renewed demand for fertiliser-phosphorus during the period of grain formation. The percentage of fertiliser-phosphorus is greater at all levels of application at the third sampling than at the second. Similar effects have been noted by Stanford & Nelson¹¹ although Gericke¹² was of the opinion that all the phosphorus required by cereals could be absorbed in the early stages of growth. Fig. 2 demonstrates that there was practically no uptake of fertiliser-phosphorus from the high-P soil between the first and second samplings, while considerable absorption of soil-phosphorus was taking place. Fertiliser-phosphorus did not appear to be fixed so rapidly in this soil as in the low-P soil, however, as a vigorous uptake took place between 54 and 94 days after sowing—probably to meet the demands of nitrogen-stimulated grain formation.

The substantial depressions in uptake of soil-phosphorus produced by the application of 40 and 80 lb. of P_2O_5 per acre as superphosphate, often giving a depression in *total* phosphorus uptake, are difficult to explain. One of the writers has observed this phenomenon repeatedly in later (unpublished) work, both with ^{32}P -labelled and normal commercial superphosphate on potatoes and oats, when yield and total phosphorus uptake were similar on radioactive and normal plots. There is an extensive literature on radiation damage in experiments with ^{32}P , and while many workers (Russell *et al.*,¹³ Russell & Martin,¹⁴ Bould *et al.*,¹⁵ Blume,¹⁶ Spinks *et al.*¹⁷) have observed differences in yield and uptake of P caused by radiation, the conclusion of most workers has been that the effects were not sufficiently large to affect the main experimental results. Penner¹⁸ recorded small but significant decreases in soil-phosphorus uptake by barley using similar activity levels to those used in this experiment and concluded that they were caused by radiation damage. It is possible, therefore, that radiation damage may have caused the depressions in the uptake of soil-phosphorus reported here. This depression became steadily more pronounced, however, as the season advanced and the radioactivity of the phosphorus applied declined. This effect has been observed repeatedly and the authors feel, therefore, that the effect mentioned is unlikely to arise from radiation damage.

Depressions in the uptake of soil-phosphorus caused by the application of phosphate fertilisers have been reported by Spinks *et al.*,¹⁷ Dean¹⁹ and McLean & Hoelscher.²⁰ The last-mentioned, working with acid soils similar to those used in this experiment, found very similar results, the uptake of soil-phosphorus by oats treated by phosphatic fertiliser being less than half of that of the control plants. The depressions of soil-phosphorus uptake by added fertiliser-phosphorus found by the writers and the workers quoted above are in sharp contrast with the findings of Dean *et al.*,²¹ Nelson *et al.*,²² and Woltz *et al.*,²³ all of whom found increases in the uptake of soil-phosphorus as well as of fertiliser-phosphorus with increase in the rate of application of phosphorus. Strzemienski²⁴ found that plants receiving phosphate fertilisers absorbed from three to eight times as much soil-phosphorus as did the control plants on deficient soils. Most of the above workers were experimenting with soils acutely deficient in phosphorus. The fact that even the soil described as 'low-P' in this experiment produced optimum grain yield under control treatment is a possible explanation of this difference between the results and those quoted above.

Nitrogen had little effect on the uptake of fertiliser-phosphorus, the marked increase in the grain being almost balanced by a reduction in the straw.

Grain yields and percentages of dry matter, fertiliser- and total phosphorus in the grain were remarkably constant for both soils. Any 'luxury' uptake of phosphorus by the plant accumulated in the straw and was not translocated to the grain.

Conclusions

(1) The final yields of oat straw produced on soils high and low in available phosphorus were unaffected by phosphate applied at rates of 40 to 160 lb. P_2O_5 per acre. The yield of grain in both soils was decreased at higher levels of application. The effect of applied phosphorus in stimulating shoot growth was noticeable only in the first four weeks after emergence.

(2) Nitrogen dressings gave increases in grain yield, particularly in the high-phosphorus soil.

(3) The general pattern of utilisation of fertiliser-phosphorus was of a higher percentage uptake from the low- than from the high-phosphorus soil. At all stages of growth and in both soils the percentage of total phosphorus in the shoot was depressed by the application of 40 lb. of P_2O_5 per acre in the form of superphosphate and increased by dressing with 160 lb. per acre.

(4) The percentage of fertiliser-phosphorus absorbed increased with rate of application in both soils, the increase being almost linear in the low-phosphorus soil.

(5) In the oats from the low-phosphorus soil the percentage of fertiliser-phosphorus fell steadily throughout the season and there was very little uptake during the period of grain formation. On the high-phosphorus soil, there was a rapid fall during the period of vegetative growth followed by an increase during grain formation.

(6) Depressions in the uptake of soil-phosphorus with increasing rates of applied phosphate were not compensated by increased uptake of fertiliser phosphorus.

(7) 'Luxury' uptake of phosphorus from the high-P soil did not give rise to increased grain formation and the extra phosphorus taken up from this soil was not translocated to the grain but retained in the straw.

(8) There was no evidence that applications of superphosphate to either soil would increase crop yield.

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STUDIES ON THE UPTAKE OF PHOSPHORUS FROM ^{32}P -LABELLED SUPERPHOSPHATE BY CROPS. II.*—Potatoes

By J. DAINTY, R. D. VERMA and K. SIMPSON

Field experiments were carried out using different amounts of ^{32}P -labelled superphosphate in combination with two rates of application of ammonium sulphate on soils high and low in 'available' phosphate. The crop was sampled at three stages of growth and analysed for dry matter and for total and fertiliser-phosphorus.

The rate of application of nitrogen had little effect on phosphorus uptake or crop growth but the uptake of soil-phosphorus from both soils was depressed by added fertiliser-phosphorus, the depression being very marked on the high-phosphate soil. Higher uptakes of fertiliser-phosphorus were not effective in increasing the final yield of tubers and the yield of dry matter was depressed on the high-phosphate soil by the application of 1.0 and 2.0 cwt. of P_2O_5 per acre (as superphosphate) compared with application of 0.25 and 0.5 cwt. The optimum yields were reached with 0.25 and 1.0 cwt. of P_2O_5 per acre on the high- and low-phosphate soils respectively.

Onset of tuber development was earlier on the low-phosphate soil. Lower rates of application of phosphorus hastened the start of tuber development and the time of maturing of the plant, giving an earlier crop, but higher rates delayed both early tuber development and the attainment of the final yield. Such effects at levels of superphosphate normally applied to the potato crop in Scotland may lead to serious reductions in yield—particularly with early potatoes.

Introduction

The experiments reported here form part of an investigation carried out over the past 14 years in the East of Scotland to examine possible economies in the present rates of application of fertiliser-phosphorus. Results of earlier work^{1, 2} have shown that, while large increases in crop yield may be obtained with moderate dressings of phosphorus in the form of superphosphate on the phosphate-deficient soils of the area, many of the soils in the main potato-growing areas are well supplied with available phosphorus and little or no crop response may be expected.³

One object of the work reported here was to investigate the uptake of applied and soil-phosphorus on two soils by sampling the crop at three stages of growth. One of the soils was fairly well supplied with 'available' phosphorus and the other, although not acutely deficient, contained much less. The soils will be referred to throughout the paper as the 'high-P' and 'low-P' soils. The second object was to determine the effect of applied phosphorus on the yield of root, shoot and tuber at three stages of growth and on the final yield of tubers.

Experimental

Soils

The soils chosen were both derived from till and are very similar in texture (sandy clay loam), both having gley horizons below 18–20 in. The high-P and low-P soils had pH values of approximately 6.2 and 6.0; available P_2O_5 260 and 25 p.p.m., respectively. The method used for estimating available P_2O_5 was that described by Kirsanov⁴ using 0.2N-HCl as extractant.

Under average climatic conditions the low-P site had an annual rainfall 5–6 in. greater than the high-P site and only about 80% of its daily sunshine. In 1955, when the experiments were carried out, the rainfall figures for the months April–September were 10.94 and 8.95 in. and the average daily hours of sunshine were 6.7 and 6.5 at the low- and high-P sites respectively. The whole period of growth at both centres was very dry and sunny but the soils had a good water reserve in April and, as previously indicated, the capillary fringe of the water table at both sites was within reach of the root systems. It was unlikely therefore that crop growth would be restricted by lack of moisture at either centre.

Treatments and layout

The treatments and layout were identical at the two sites. Superphosphate at five rates, equivalent to 0, 0.25, 0.5, 1.0 and 2.0 cwt. of P_2O_5 per acre, was combined with ammonium

* Part I: preceding paper

sulphate at two rates, 0.5 and 1.0 cwt. of N per acre. The ten treatment combinations were replicated five times in randomised blocks at each centre. All plots received a basal dressing of potassium chloride equivalent to 168 lb. of K_2O per acre. Each plot consisted of 9 drills, 27 in. wide and 20 ft. long. ^{32}P -labelled superphosphate was used at three rates only (0.25, 0.5 and 1.0 cwt. P_2O_5 per acre) and was applied to the centre drill of the appropriate plots.

Handling of radioactive materials

Approximately 6 kg. (0.7 curie) of ^{32}P -labelled superphosphate was handled in this and associated pot experiments on oats (see preceding paper⁵). The fertiliser was applied within two days of manufacture and the approximate amounts of ^{32}P per 12 feet of drill were 4.8, 9.6 and 19.2 mc for the three rates of application.

Planting, sampling and harvest

A second-early variety, Craigs' Royal, was used to ensure that plants would mature early and thus ensure practicable counting rates of the ^{32}P . Selected seed tubers within 5 g. of the mean seed weight (90 g.) were used for the ^{32}P -treated drills. Stock seed was used and all tubers were sprouted before planting. Fertilisers were applied in the drill and the seed was hand-planted accurately at 1-ft. spacing before ridging up on the same day.

The plants were sampled at 8, 12 and 17 weeks after planting, i.e. shortly after the start, in the middle and at the end of tuber development. In each radioactive sub-plot, pairs of plants were sampled at random at each sampling. The two end plants were used as guard plants and not sampled. At later samplings, plants adjacent to pairs already sampled were excluded. In all cases, the whole plant was lifted by two operators, using forks, and transferred with the soil to large waterproof paper bags. All soil was removed by means of a water spray on a $\frac{1}{4}$ -in. mesh sieve. After being washed, the two plants from each plot were combined and left overnight for surface water to drain away. The following day the shoot, root, and tubers were separated and weighed. In all cases the whole root system was used for determination of dry matter, but at the second and third samplings it was necessary to sub-sample the shoot and tubers before drying.

Analytical methods

Total phosphorus and ^{32}P were determined by the method described in the preceding paper.⁵

Results and discussion

There was no significant difference between the effects of the two rates of application of nitrogen on the yield of root, shoot and tuber or on the uptake of phosphorus at either centre, nor was there a significant interaction between nitrogen and phosphorus treatments. The results for these treatments have therefore been combined in Table I a-d.

The most striking effect of phosphate treatments on yield was in the shoot during the early part of the season. On the low-P soil in the first eight weeks of growth, the dressing with 2 cwt. of P_2O_5 per acre had produced 5 times as much shoot as the control and more shoot growth than the equivalent rate on the high-P soil. The stimulation of shoot growth was continuous up to the highest level of application on both soils despite the slight depression in root yield at this level, on both soils, compared with the 1.0 cwt. per acre level.

The very vigorous shoot growth stimulated by high phosphate treatments early in the season had the effect in both soils of delaying the development of tubers. On the low-P soil the lower rates of application gave early increases in tuber yield but the 2.0 cwt. per acre rate was much less effective. The increases over control were, respectively, 17, 36, 31 and 11 cwt. per acre for the 0.25, 0.5, 1.0 and 2.0 cwt. P_2O_5 per acre rates. On the high-P soil at the 8-weeks stage the tuber yield was only about one-third of that on the low-P soil for all treatments. Climatic conditions would have suggested that tuber development would start earlier at the high-P site. The two sites were chosen for their general similarity in soil properties and, in the season concerned, weather conditions were similar at the two sites. There were, however, 2.25 in. more rain at the low-P site which might have been expected to delay tuber development at this stage. It seems possible that the very high uptake of phosphorus from the high-P soil and from

Table I

(a) Yield of fresh shoot, root and tuber (cwt. per acre) on the three dates of sampling and the final yield of tubers

Weeks between planting and sampling	Part of plant	Low-phosphate soil						High-phosphate soil					
		P_2O_5 applied, cwt./acre					L.S.D.	P_2O_5 applied, cwt./acre					L.S.D.
		Nil	0.25	0.5	1.0	2.0		Nil	0.25	0.5	1.0	2.0	
8	Shoot	19.8	47.2	65.1	82.6	94.0	15.1	53.3	78.8	76.1	88.0	89.9	20.3
	Root	5.0	5.8	8.1	8.8	6.9	1.84	6.7	8.6	8.8	10.3	9.3	—
	Tuber	33.5	50.3	69.3	64.3	44.5	17.1	11.4	17.1	19.4	25.5	19.8	7.8
12	Shoot	45.7	69.7	98.6	113.1	126.0	26.0	113.1	141.6	132.1	127.5	139.4	N.S.
	Root	6.9	8.9	10.8	11.3	9.9	2.08	9.4	10.1	9.6	9.1	8.8	N.S.
	Tuber	100.9	166.4	208.6	225.4	196.5	28.5	186.2	233.0	205.2	200.3	199.9	36.0
17	Tuber	172.5	269.9	289.4	294.7	287.5	40.4	295.0	337.7	339.6	306.1	302.7	30.8
19-20	Tuber	192.0	264.4	285.8	305.6	282.8	38.6	292.0	353.0	335.2	339.4	327.2	35.8

(b) Effect of superphosphate treatments on the percentage of P_2O_5 in the dry matter of shoot, root and tuber

8	Shoot	0.50	0.60	0.65	0.70	0.98	0.11	0.88	1.08	1.22	1.20	1.40	0.22
	Root	0.28	0.35	0.37	0.49	0.81	0.074	0.53	0.74	0.76	0.86	1.10	0.15
	Tuber	0.32	0.41	0.45	0.52	0.77	0.059	0.60	0.76	0.85	0.88	0.89	0.15
12	Shoot	0.54	0.40	0.38	0.36	0.45	*	0.55	0.53	0.61	0.59	0.59	*
	Root	0.34	0.27	0.22	0.25	0.38	*	0.47	0.50	0.50	0.48	0.64	*
	Tuber	0.35	0.29	0.30	0.32	0.40	*	0.50	0.44	0.47	0.50	0.53	*
17	Shoot	0.39	0.28	0.31	0.30	0.32	0.049	0.36	0.34	0.34	0.35	0.33	N.S.
	Root	0.31	0.23	0.22	0.24	0.26	0.047	0.26	0.26	0.27	0.27	0.29	N.S.
	Tuber	0.33	0.28	0.29	0.32	0.35	0.030	0.39	0.34	0.45	0.49	0.45	0.060

(c) Total uptake of P_2O_5 (lb. per acre) in shoot, root and tuber as affected by superphosphate treatments

8	Shoot	1.53	3.46	5.02	6.41	9.50	1.01	4.78	7.72	8.21	8.28	9.87	1.77
	Root	0.29	0.42	0.53	0.74	1.09	0.10	0.68	1.09	1.16	1.31	1.55	0.31
	Tuber	2.01	3.95	5.76	6.36	5.76	0.98	1.14	2.05	2.69	3.75	2.79	0.57
12	Shoot	3.01	2.84	4.03	4.24	6.16	*	8.57	9.41	10.56	9.94	10.68	*
	Root	0.34	0.35	0.31	0.25	0.58	*	1.03	1.09	1.05	0.95	1.31	*
	Tuber	7.59	11.73	14.58	17.69	18.14	*	21.17	24.23	22.79	23.78	25.78	*
17	Shoot	2.17	1.84	2.28	1.98	3.20	0.45	4.31	3.81	4.32	4.37	4.23	N.S.
	Root	0.37	0.31	0.32	0.35	0.39	N.S.	0.93	0.98	1.20	1.03	1.27	N.S.
	Tuber	13.58	18.28	20.68	23.24	26.24	3.47	29.84	27.65	37.48	36.51	33.71	5.21

(d) Uptake of fertiliser P_2O_5 (lb. per acre) in shoot, root and tuber

8	Shoot	—	1.56	2.86	4.29	—	0.29	—	1.93	3.28	3.97	—	0.56
	Root	—	0.15	0.27	0.45	—	0.06	—	0.21	0.35	0.51	—	0.058
	Tuber	—	1.70	3.34	4.32	—	0.42	—	0.43	0.97	1.54	—	0.18
12	Shoot	—	0.74	1.61	2.46	—	*	—	1.32	2.53	3.98	—	*
	Root	—	0.07	0.11	0.12	—	*	—	0.11	0.21	0.31	—	*
	Tuber	—	3.52	7.14	11.32	—	*	—	3.87	6.61	9.27	—	*
17	Shoot	—	0.35	0.75	0.83	—	0.09	—	0.42	0.73	1.14	—	0.11
	Root	—	0.05	0.07	0.12	—	0.015	—	0.09	0.17	0.24	—	0.031
	Tuber	—	5.48	10.34	14.41	—	1.73	—	4.15	8.62	13.51	—	1.46

L.S.D. = lowest significant difference ($P = 0.05$)

N.S. = not significant

* Composite samples

the plots receiving 2.0 cwt. of phosphate per acre on the low-P soil (Table Ic) was responsible for delaying tuber development at this stage. The particularly high percentage of phosphorus in the shoot at the first sampling (Table Ib) lends support to this idea. On the low-P soil this percentage came up to the level of the shoots on the high-P soil only at the 2.0-cwt. per acre rate of application which delayed tuber development compared with lower rates of application.

The period between 8 and 12 weeks after planting was very dry and sunny (9 hours daily

average at both centres). During this period the high-P site received 1 in. more rainfall than the low-P site. Despite this, only the control and the 0.25-cwt. rate of application at the high-P centre had produced a greater yield of tubers than the equivalent treatments on the low-P soil. The higher rates produced about the same yield on both soils. It was only at the third sampling and in the final yield that all plots on the high-P soil yielded larger crops of tubers than the corresponding plots on the low-P soil.

The final yield figures indicate that, at maturity, the optimum dressings of P_2O_5 on the high- and low-P soils were 0.25 and 1.0 cwt. per acre. If, as is the normal practice, the haulms had been sprayed with sulphuric acid as a blight-prevention measure at about the time of the third sampling it is doubtful if more than 0.25–0.5 cwt. per acre would have been profitable even on the low-P soil.

The percentage of total phosphorus in all parts of the plant, as shown in Table Ib, increased very sharply with fertiliser increments at the first sampling. At the 12- and 17-week stages the high-P soil had supplied sufficient phosphorus to maintain a high and fairly uniform percentage of phosphorus in shoot, root and tuber irrespective of fertiliser treatment. On the low-P soil, however, the phosphorus content of the shoot and root fell rapidly as the requirements of the tubers increased. This was particularly noticeable with 0.25, 0.5 and 1.0 cwt. of P_2O_5 per acre, whereas the 2-cwt. level maintained a high concentration, particularly in shoot and tuber throughout the season. It may be significant that this treatment gave no extra yield over lower applications, and attained its maximum yield at a later date, indicating that phosphorus was present in luxury amounts. This was the case also on the high-P soil for all levels of application over 0.25 cwt. per acre. On this soil, the highest yield of tubers at the third sampling (Table Ia) was produced by 0.25 cwt. of P_2O_5 per acre which gave tubers with the lowest phosphorus content. The best yielding crops on the two soils contained 0.32 and 0.34% of P_2O_5 in the dry matter of the tubers, suggesting a possible optimum content. This was exceeded by 40–50% at the 1.0- and 2.0-cwt. levels on the high-P soil, where tuber development was delayed. The phosphorus contents of root, shoot and tuber were always higher on the high-P soil than on the low-P soil. In the shoot at the first sampling it reached the very high level of 1.4% of P_2O_5 at the highest level of application (non-radioactive) which depressed the yield at the third sampling compared with lower rates (0.25 and 0.5 cwt.).

With few exceptions, there was a steady decrease in the amount of phosphorus per acre contained by shoot and root throughout the season on the low-P soil. The amount of phosphorus absorbed by the whole plant increased throughout the season and considerable absorption, mostly of soil phosphorus, took place during the later stages of growth (Table Ic). The amount of phosphorus taken up by the tubers increased at a greater rate than the amounts in the shoot and root decreased, the balance being made up in the later stages of growth largely from soil phosphorus.

On the high-P soil, associated with the later onset of tuber development, there was an increase in phosphorus uptake by the shoot between the first and second samplings, translocation to the tubers taking place later. The phosphorus uptake of all parts of the plant was at all samplings greater on the high-P soil than on the low-P soil except at the first sampling of tubers.

Treatments giving optimum tuber yield on the low-P and high-P soils respectively (1.0 and 0.25 cwt. of P_2O_5 per acre) produced a crop containing, in the tubers, 23 and 27 lb. of P_2O_5 per acre. Considerably higher levels of uptake—33, 36 and 37 lb. per acre—all gave lower yields at the third sampling.

Extra increments of fertiliser up to the highest level increased the uptake of fertiliser phosphorus in all parts of the plant on both soils, at all stages of growth (Table Ic). As expected, there was a much lower percentage of the total uptake derived from fertilizer on the high-P soil. The percentage of phosphorus derived from fertiliser fell steadily in all parts of the plant as the season progressed. The fall was sharpest in the roots and was also more marked at the lower levels of application. For example, between the first and third samplings for shoots on the low-P soil the fall was from 45 to 19% at the 0.25-cwt. level and from 67 to 42% at the 1.0-cwt. level. For tubers on the high-P soil the fall was from 21 to 15% at the 0.25-cwt. level and from 41 to 37% at the 1.0-cwt. level. These facts suggest that fertiliser-phosphorus was

becoming rapidly less available to the plant in both soils as the season progressed. The indications were that the availability of applied phosphorus was declining at a similar rate in the two soils.

The expected stimulus of soil phosphorus uptake observed by many other workers⁶⁻⁸ was not found, apart from a slight stimulation on the low-P soil at the first sampling. In later samplings on this soil and at all stages on the high-P soil, the uptake of soil phosphorus was depressed. On the high-P soil these depressions in uptake were very substantial (Fig. 1) giving rise to uptakes of total phosphorus only slightly higher than that for the control. Similar

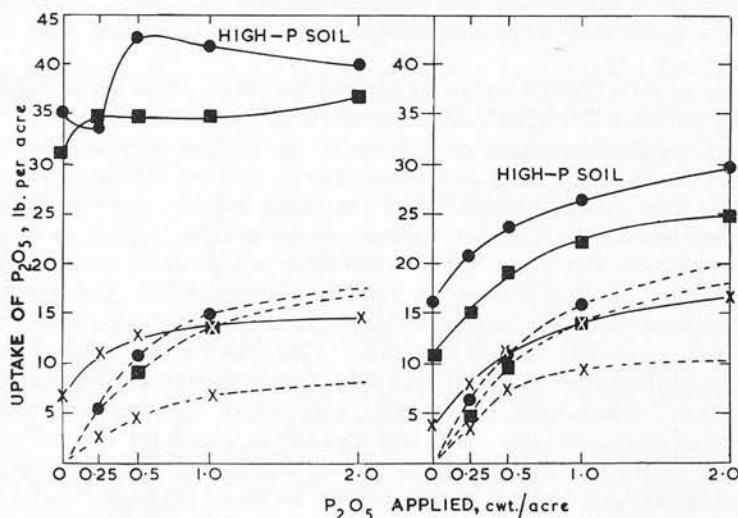


FIG. 1.—Uptake in lb. per acre of total and fertiliser P_2O_5

× after 8 weeks
□ after 12 weeks
● after 17 weeks

— total P_2O_5
- - - fertiliser P_2O_5

depressions have also been observed with oats⁵ and by Spinks *et al.*,⁹ Dean¹⁰ and McLean & Hoelscher.¹¹ In most cases, where stimulation of uptake of soil phosphorus by addition of fertiliser-phosphorus occurred, the soils were acutely deficient in phosphorus. In this investigation the low-P soil was capable of supplying sufficient phosphorus for 10 tons of potatoes per acre without any fertiliser-phosphorus. It may be, therefore, that on the more deficient soils of the area, stimulation of uptake of soil-phosphorus by fertiliser could be found.

The possible effects of radiation damage and isotopic exchange on the results presented above have been reviewed in the preceding paper⁵ and more thoroughly by Mattingley.¹² The writers do not feel that either effect could invalidate the conclusions made above.

The most striking observation was that, on both soils, while the lower rates of applied phosphorus hastened the early development of tubers and also the attainment of the final yield, the higher rates of application (Fig. 2) delayed both of these stages of growth. In all cases this delay in maturity was associated with very high uptake of phosphorus. In fact, on the high-P soil the yield at the third sampling was inversely proportional to the phosphorus content of the crop. On the low-P soil, at the second sampling, the amount of dry matter (tubers) from the plot receiving 2.0 cwt. of P_2O_5 per acre was 1000 lb. per acre less than from the 1.0-cwt. plot. As is shown in Fig. 2, production of dry matter on this soil was complete at the third sampling, no further addition being made in the final yield, and at this stage only was the yield of dry matter from the 2.0-cwt. rate equal to those from the 1.0- and 0.5-cwt. of P_2O_5 per acre rates.

On the high-P soil at the second sampling, the lowest rate of application (0.25 cwt. of P_2O_5 per acre) had produced 700–800 lb. per acre of dry matter more than all higher rates. At the third sampling, both the 0.25- and 0.5-cwt. rates gave 1200–1300 lb. per acre more than the higher rates and 1000 lb. more than the control. The final yield curve in Fig. 2 indicates that the 1.0- and 2.0-cwt. rates produced more dry matter between the third sampling and harvest,

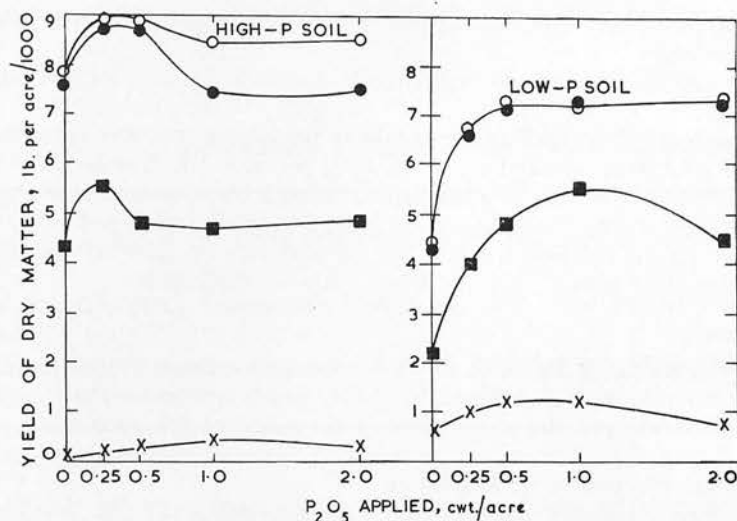


FIG. 2.—Yield of dry matter (tubers) in lb. per acre/1000

× 8 weeks after planting
■ 12 weeks after planting

● 17 weeks after planting
○ final yield

but did not reach as high a level of production of dry matter as the lower rates of application. The results in Fig. 2 might, of course, be explained as a balance of two opposing effects between phosphorus response and radiation damage. Numerous data from field experiments, however, carried out with unlabelled superphosphate (now being prepared for publication), strongly suggest that this is not the case.

From the above observations it seems that phosphorus present in excessive quantities in the plant tissue has interfered in some way with the ripening processes of the plant. This delay in tuber production and ripening by high rates of application of superphosphate is very important to potato growers in Scotland. It is usual for maincrop potatoes to be 'sprayed down' before the normal senescence of the haulms and under the experimental conditions this would have led to the highest yields being produced by only 0.25 cwt. per acre of P_2O_5 as superphosphate on the high-P soil and 0.5 cwt. per acre on the low-P soil. Most farmers in the potato-growing areas apply considerably more superphosphate than this. Recent fertiliser surveys in Aberdeenshire, Angus and East Lothian¹³ show that 100% of maincrop potatoes received fertiliser at rates which are equivalent to average dressings of 0.95, 0.98 and 1.14 cwt. of P_2O_5 per acre for the three counties respectively. In addition to this, between 60 and 80% of maincrop potatoes received farmyard manure at rates between 12 and 17 tons per acre which would supply at least 70–80 lb. of P_2O_5 per acre. Many of the soils have higher available phosphorus contents than the high-P soil in this experiment. Simpson³ has demonstrated that, under normal farm practice, lack of response to applied phosphorus on these soils occurs over widely varying soil and climatic conditions and that depressions in yield may arise from high rates of application. It is felt, therefore, that there is a good case for a considerable reduction in the rate of application of phosphate fertilisers to the potato crop in Scotland.

The problem of later tuber development with high rates of applied phosphorus may be even more important in the early-potato growing areas where, according to a recent fertiliser survey in Ayrshire,¹³ all early potatoes receive fertiliser at an average rate equivalent to 1.92 cwt. of P_2O_5 per acre as superphosphate. In addition 55% of the growers use an average of 18 tons of farmyard manure per acre. It seems that such rates of application are completely unjustified where early-tuber production is desirable.

Conclusions

(1) The optimum final yield of potatoes was reached at levels of 0.25 and 1.0 cwt. of P_2O_5 per acre as superphosphate in the high-P and low-P soils respectively. Uptake of fertiliser-phosphorus in both soils increased with each increment of fertiliser, but decreased steadily

throughout the season. There was little uptake of fertiliser-phosphorus from either soil after 12 weeks from planting.

(2) Uptake of soil-phosphorus was substantially depressed by additions of fertiliser phosphate on both soils.

(3) There appeared to be a limit to the uptake of phosphorus per acre required to produce the maximum crop of tubers—around 23–27 lb. P_2O_5 per acre. Higher levels of uptake than this on both soils delayed both early-tuber formation and the attainment of the final yield. These levels of uptake occurred with 2.0 cwt. of P_2O_5 per acre on the low-P soil and with 0.5, 1.0 and 2.0 cwt. per acre on the high-P soil. The 1.0- and 2.0-cwt. rates on this soil gave lower final yields of dry matter tubers than the lower rates of application.

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THE COLOUR OF COOKED CURED PORK.

III.*—Distribution and Relationship of Pigments, pH and Cysteine

By H. C. HORNSEY

Variations in the pigment concentration of fresh pork, in different muscles or in the same muscle, lead to variations in the colour of the cooked cured pork. Two-fold variations were found for different muscles, these being due almost wholly to myoglobin, and not to haemoglobin.

The relationships between pH, cysteine, cystine, total pigments, nitroso pigments, and stability of colour have also been investigated, pH appearing to have opposite effects in the fresh pork and the cooked cured pork.

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THE EFFECT OF RATE OF APPLICATION OF SUPERPHOSPHATE ON THE GROWTH

AND YIELD OF POTATOES¹

by

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The effect of various rates of application of superphosphate between 0 and 4 cwt. P_2O_5 per acre on the yield and growth of potatoes was studied in a very wet season (1954) and a dry one (1955). Fresh and dry weight of shoot, root and tuber, P_2O_5 uptake in root, shoot and tuber, population, shoot height, and number of sprouts per hill were all observed at three stages of growth. Final yields of ware, seed and chits were recorded. Although shoot yield was stimulated by dressings up to 2 cwt. P_2O_5 per acre on the "low P" soils, root and tuber dry matter were not further increased by dressings above 0.66 cwt. P_2O_5 per acre. On "high P" soils superphosphate applications had little effect on shoot yield but delayed tuber development and depressed the final yield of tuber dry matter.

There was a positive correlation between P uptake and yield of tuber dry matter on the low P soils and a negative one on high P soils. It is suggested uptake of P_2O_5 per acre at the 12 week stage for optimum tuber production was 30 - 35 lb. per acre and that phosphorus toxicity occurs at higher levels of uptake.

INTRODUCTION

The experiments reported here form part of a long term investigation being carried out in the East of Scotland on the effect of phosphate fertilisers on several crops. Results of earlier work; Smith and Simpson (1), Simpson (2) have shown that, while large increases in crop yield may be obtained with moderate dressings of phosphorus in the form of superphosphate on the phosphate deficient soils of the area, many of the soils in the main potato growing districts are well supplied with available phosphorus and there is little or no crop response

to applied phosphorus (3). More intensive work, Dainty et al. (4), has shown that a reduction in crop yield and delay in tuber production may arise from the application of more than about 1.0 cwt. P_2O_5 per acre as superphosphate on soils high in "available" phosphorus.

The object of the present work was to determine the effect of various applications of superphosphate on the fresh and dry weight and the P_2O_5 content of shoot, root and tuber, plant population, number of tubers per plant, shoot height, number of sprouts per hill and "density" of shoots at three stages of growth, with a view to establishing, if possible, the reasons for the delay in tuber production and the decreases in yield associated with high dressings of superphosphate previously reported (5). The three stages of growth selected coincided approximately with the onset, middle and end of the period of tuber formation.

A brief description of the soils from the five sites is given in Table I.

<u>Soil No.</u>	<u>Year</u>	<u>Av. pH. value</u>	<u>Available P_2O_5 p.p.m.</u>	<u>Description of parent material</u>
1.	1954	5.9	25	Clay till
2.	1954	6.0	220	Old raised beach
3.	1954	7.5	600	Fluvio-glacial sand and gravel
4.	1955	5.6	50	Sandy clay till
5.	1955	6.2	260	Sandy clay till

The method used for estimating available phosphorus was that described by Kirsanov (6) using N/5 HCl as solvent.

For convenience of reporting the soils from centres 1 and 4 will be referred to as "low P" and from centres 2, 3 and 5 as "high P" soils.

Climate.

The seasons of 1954 and 1955 were sharply contrasting in both rainfall and

hours of sunshine. Average data from two local meteorological stations showed, for the months of April to October, 28.8 and 9.9 inches of rain in 1954 and 1955 respectively. The corresponding figures for the hours of sunshine per day were 4.2 and 5.7. 1954 was therefore a very wet season with little sunshine while 1955 was rather dry and sunny. In the peak period of growth in 1955 there were 9 hours of sunshine daily at both centres.

Treatment and Design.

In 1954 the design of all three experiments consisted of 4 randomized blocks of six treatments - superphosphate at rates equivalent to 0, 0.33, 0.66, 1.0, 2.0 and 4.0 cwt. P_2O_5 per acre. In 1955 the highest treatment was omitted and at both centres five treatments were used 0, 0.25, 0.5, 1.0 and 2.0 cwt. P_2O_5 (cwt.) per acre as superphosphate. Basal dressings of 5 cwt. ammonium sulphate and 2.5 cwt. potassium chloride per acre were used in all experiments. This practice was based on the results of a previous series of experiments reported elsewhere (1).

Planting, Sampling and Harvest.

In all cases the seed potatoes were planted at 1 foot spacing in drills 27" wide into which the fertiliser had previously been placed. In 1954 unsorted stock seed potatoes were planted but in 1955 the smallest and largest seed were discarded giving an average seed weight of 90 g. with the seed planted varying between 75 and 105 g. The varieties used were Kerr's Pink (1954) and Craigs' Royal (1955).

Measurements on plants and sampling of selected roots for yield estimates and analysis were carried out at times as near as possible to 8, 12 and 16 weeks after planting, i.e. shortly after the start, in the middle and towards the end of tuber development. A fourth sampling of the plants was also carried out at harvest when the shoot was usually dead or senescent. For height measurements 10 plants were selected at random in each plot and labelled on one of the

main shoots. This particular shoot was measured at all 3 observation periods. In 1954 dry weight data and samples for analysis were obtained from a composite sample of 2 plants randomly selected from each plot. In 1955, however, one drill was set apart for sampling and pairs of plants were sampled at random. In all cases, the whole plant was lifted by two operators using forks, and transferred with the soil into large waterproof paper bags. All the soil was removed by means of a water spray on a $1/4$ inch mesh sieve. After washing, the two plants from each plot were combined and left overnight to allow surface water to drain. The following day the roots, shoots and tubers were separated and weighed. In all cases the whole root system was used for dry matter determination, but at the second and third sampling it was necessary to subsample the shoots and tubers before drying. Total phosphorus in each sample was determined by the method described by Piper (7). The digests were filtered, made up to 100 ml. in graduated flasks and suitable aliquot parts taken for analysis. The plants were allowed to mature and at harvest the whole crop was lifted by hand and dressed over $1\ 1/4$ " and $2\ 1/4$ " riddles to separate ware, seed and chats, the yields of each size group being recorded.

RESULTS

The population and number of sprouts per hill were not significantly affected by superphosphate treatments except for a depression on population at the 4.0 cwt. per acre level of P_2O_5 application at centre 2. On the "low P" soils in both seasons there was a steady rise, with increasing rates of phosphate application, in the shoot height and number of tubers per plant. At the three "high P" centres the effect of treatments on shoot height and number of tubers per plant was generally non-significant. The results for the above observations have not been presented because they follow a regular and expected pattern. Nor are the fresh weight figures

recorded here since they were of doubtful accuracy following the washing procedure for the removal of soil. In general, however, the percentage of dry matter in the different parts of the plant was steadily reduced by successive additions of superphosphate.

Table II shows the effect of superphosphate treatments on the yield of dry matter of shoot and root at the 8, 12 and 16 week stages of growth. On the low P soils (1 and 4) the yield of shoot was greatly increased by additions of superphosphate. In 1955, the effect was most marked during the early part of the season when successive additions of fertiliser phosphate gave significant increases in yield up to the 1 cwt. level, this treatment yielding about three times as much dry matter as the control at the 8 week stage. Later in the season the 2 cwt. per acre rate of application became significantly superior to the 1 cwt. treatment but the general effect of fertiliser treatments on shoot growth was less marked. In the 1954 season the effect of applied phosphate was small in the early stages of growth but became large by the 16 week sampling when the 1 cwt. P_2O_5 per acre dressing had produced about four times as much shoot dry matter as control. This may be due to the wet season extending the period of availability of fertiliser phosphorus. The effect of superphosphate on shoot growth on the "high P" soils (centres 2, 3 and 5) was not so great. Small dressings (up to 0.66 cwt. P_2O_5 per acre) increased shoot yield at the 4 week stage, but no significant differences were found after 16 weeks. At centre 2 a significant depression in shoot yield was produced by the 4.0 cwt. per acre rate of application and lasted throughout the season. This depression was associated with a reduction in the number of plants per acre and a lowering in the yield of root material.

^{But}
~~By~~ the effect of added superphosphate on root development was not so great. At centres 1 and 3 there was no stimulation of growth at the 8 week stage and on

the "very high P" soil - centre 3, the only increase was given by the 0.33 cwt. per acre rate of application at the 12 week stage. In no case was there any significant increase in root weight produced by additions of superphosphate at rates greater than 1.0 cwt. P_2O_5 per acre. In fact further addition of phosphorus often depressed root growth, particularly at centres 2 and 3 in the wet season of 1954. At centre 3 (very high P) the depression became progressively severe throughout the season and at the 16 week stage, all dressings above 0.33 cwt. per acre gave significantly lower root yields than control, the 4.0 cwt. rate of application giving further significant depression and producing little more than half of the control yield. This depression was reflected in the tuber yield (Fig. I).

Figure I shows the effect of superphosphate on the yield of dry matter in the tubers at the 12 week, 16 week and maturity stages at all five centres. On the low P soils optimum final yields were obtained at the 0.66 and 0.5 cwt. P_2O_5 per acre levels for centres 1 and 4 respectively. At the three high P centres (numbers 2, 3 and 5), 0.66, 0.33 and 0.25 cwt. P_2O_5 per acre gave the best yields of dry matter in the final crop. Even on the low P soil (No. 1) further increments in superphosphate application gave lower final yields of dry matter and on the three high P soils significantly lower amounts of dry matter were produced by the 2 and 4 cwt. and at centre 5 by the 1 cwt. rate of application. More important, however, is the pattern of tuber development. At all centres, particularly centre 3, early tuber development was stimulated by small dressings of superphosphate. In the three high P soils only the lowest rate of application (0.25 - 0.33 cwt. P_2O_5 per acre) was effective in doing this and higher dressings often delayed tuber development. This effect was most noticeable on the very high P soil at centre 3 where, at the 16 week stage the 2.0 and 4.0 cwt. rates of P_2O_5 per acre had produced 600 and 2000 lb. less dry matter per acre than the 0.33 cwt.

level and the 4.0 cwt. rate had produced significantly less than control.

Figure 2 shows the uptake of P_2O_5 in lb. per acre by shoot and (root + tuber) as affected by superphosphate treatments at the 8, 12 and 16 week stages. The uptake in the roots was usually only a small proportion of the total uptake, the only exception being at centre 3, 1st sampling, where almost half of the amounts shown for (root + tuber) was contained by the roots. There was no obvious effect of season on the uptake of phosphorus. The uptake in both parts of the plant increased steadily with each addition of fertiliser on the two low P soils (centres 1 and 4). At both centres there was vigorous uptake by the shoot on superphosphate treated plots before the 8 week stage, followed by a steady decline in shoot phosphorus as the tubers developed. The phosphate uptake of the tubers increased throughout the season at both centres. From a comparison of Fig. I and II for these two centres it is clear that, while phosphorus uptake goes on increasing for each increment in fertiliser application, the yield of dry matter of tubers does not. At centre 1 the optimum yield is obtained, at all stages, at the 0.66 cwt. P_2O_5 per acre level of application and at centre 4 the 0.5 cwt. level gives the optimum yield except at the second sampling where the 1 cwt. treatment gives a further increase. It is plain, therefore, that the higher rates of application are giving rise to luxury consumption of phosphorus which is giving no further increase in yield. Indeed, where the level of phosphorus absorption is highest at centre 4 there was a delay in tuber development. At centre 1, the amount of P_2O_5 absorbed for optimum tuber dry matter production at the three samplings was 14.5, 22.2 and 31.4 lb. per acre. The 4.0 cwt. per acre treatment gave rise to uptakes of 34.3, 40.1 and 50.5 lb./acre of P_2O_5 with no further increase, in fact a slight decrease, in final tuber yield. Similar effects may be seen for centre 4.

At the "very high P" centre 3 there was a very high phosphorus uptake in the shoot at the 8 and 12 week stages. At the 12 week stage in particular,

superphosphate treatments stimulated the uptake to a very high level, (24.2, 29.0 and 35.5 lb. P_2O_5 per acre for the 1, 2 and 4 cwt. rates of application). While, however, the shoot phosphorus at the lower levels of application, 0.33 and 0.66 cwt. P_2O_5 per acre, was effectively transferred to the tubers (as shown by the 3rd sampling shoot graph - Fig. II) and was used in increasing the yield of tubers (Fig. I) this did not occur at higher levels of application. The high phosphorus uptake from these treatments (1, 2 and 4 cwt. P_2O_5 per acre) was in fact associated with a very marked delay in tuber production and a reduction in final yield. At the other high P centre 2, there was never more than 16 lb. P_2O_5 per acre in the shoot at any sampling and the amount was not significantly affected by treatments. The amount of phosphorus in the tubers was, however, increased by all treatment and where this increase was significant at the 12 and 16 week stages it was associated with a delay in tuber development and with a depression in final yield of tubers.

The moderate P soil, centre 5, shows a similar pattern to the two low P for shoot uptake, the phosphorus being efficiently transferred to the tubers as the season progressed, but only at the first sampling was the uptake of phosphorus in the shoot stimulated by the 0.25 cwt. P_2O_5 per acre superphosphate treatment with not further significant increases from higher dressings. The early tuber development at the 0.25 cwt. P_2O_5 per acre level and the optimum final yield were associated with the lowest P_2O_5 content. The 1.0 and 2.0 cwt. levels of application depressed the final yield when compared with the 0.25 cwt. level.

With the above observations in mind, it was decided to correlate the uptake of phosphorus at all centres and under all treatments with the final yield of dry matter (tubers). The uptake figures selected for presentation represent the 12 week sampling where the amount of phosphorus uptake was at or around its peak. Similar correlations were found using the uptake data at the 8 and 16 week stages.

Figure IV shows the uptake in lb. P_2O_5 per acre plotted against the final yield of tubers. The two groups of soils show remarkably different effects. The correlation coefficients for the two groups of points were:-

Low P. soils:- $r = + 0.642$ ($n = 11$ Sig. $P = 0.05$)

High P. soils:- $r = - 0.546$ ($n = 17$ Sig. $P = 0.05$)

The high P soil correlation coefficient was obviously reduced considerably by the two ringed points. These points represent the 4.0 cwt. P_2O_5 per acre treatment at centres 2 and 3, both of which caused serious restriction in root development throughout the season. As these treatments were outstanding in this respect it is thought that there is good reason for eliminating these two points. The resultant correlation coefficient is $r = -0.787$ ($n = 15$ Sig. $P = 0.001$).

The potato crop in S.E. Scotland is grown largely for seed production and it is important to know the effect of add^{ed} superphosphate on the production of ware (2 1/4"), seed (1 1/4" - 2 1/4") and chats (1 1/4"). All the crops were graded and Fig. III shows the effect of superphosphate treatments on the yield of ware, seed and chats. It is immediately obvious that the proportion of ware to seed is much greater in the dry 1955 season than in 1954 which was cool and wet. In 1954, 60-75 per cent of the crop was in the seed size range whereas in 1955 only 30-45 per cent by weight was seed. The seed yield at the two low P centres 1 and 4, was increased significantly by all superphosphate treatments compared with control. In 1954 the increase was much higher at the lower levels of application (5.8 tons of seed from 0.66 cwt. P_2O_5 per acre compared with 1.1 tons in 1955). At centre 5 (1955), however, further applications of superphosphate, up to 2.0 cwt. P_2O_5 per acre, continued to increase seed yield whereas there was not further increase at centre 1. The effect on ware yield was the reverse, with very little effect of superphosphate additions in 1954 and large effects up to the 1.0 cwt. P_2O_5 per acre level in 1955.

The effect of added superphosphate on yield at the three high P centres was non-significant, except for a depression in ware yield at centre 3 by the 2.0 and 4.0 cwt. P_2O_5 per acre rates and a depression in seed yield at the 4.0 cwt. level at centre 2.

DISCUSSION

The results are little affected by the number of plants per acre and the number of sprouts per hill, the latter being unaffected by treatments. The population was affected only on the very high phosphate soil where it was significantly depressed by the 4.0 cwt. P_2O_5 per acre rate of superphosphate.

Stimulation of shoot and root development by add superphosphate on the low P soils was considerable, the shoot yield being increased by successive additions up to 1.0 or even 2.0 cwt. per acre throughout the season. These yield increases were accompanied by similar increases in shoot height. The extra shoot yield produced by dressings higher than 0.66 cwt. P_2O_5 per acre did not give rise to extra tuber production. In fact in the wet season of 1954 it was associated with a slight depression in tuber yield.

Root yield on the low P soils was not further stimulated by applications above 0.66 cwt. per acre, the level at which dry matter yield of tubers was also at an optimum. The number of tubers per plant showed consistent increases with added fertiliser significant up to the 1.0 cwt. level of application, which would largely account for the increases in yield. In the high P soils, on the other hand there was little stimulation of root and shoot growth by any rate of application. At two of the centres the highest rate of application brought about serious restriction of the root system lasting throughout the season. On this group of soils the number of tubers per plant was not increased by added superphosphate.

The effect of season showed itself chiefly in the yield of shoot material, which was considerably higher in the wet season (1954), in the gross yield of

tubers and the ratio of ware to seed size tubers produced. Tuber yields were higher on both high and low P soils in the dry season of 1955 than in the wet year, 1954, both on control and treated plots. This agrees with the previous observations (2) that in wet seasons there are bigger responses to phosphate fertilisers on deficient soils, possibly associated with lower availability of soil phosphorus. The higher 1955 yields were obtained not by increases in the gross number of tubers but by an increase in the number reaching ware size giving considerably higher yields of ware at the expense of the seed fraction. It also appears that the shoot yield in the wet season was increased at the cost of tuber production, the plant maturing without producing many large tubers.

The depressions in yield produced by the higher phosphorus treatments at the very high P centre and by the 2.0 cwt. rate at centre 4 were almost entirely accounted for by reductions in ware yield, but at centre 2 the depression was mainly in the seed fraction.

Low responses in final yield of tubers to applied superphosphate on high P soils are shown in Fig. I as in a previous series of experiments (3). High phosphate status is common in the soils of the potato growing areas. In 1956, 86 and 76 per cent of all soil samples taken from Angus and E. Lothian respectively were "satisfactory" and 54 and 55 per cent high in "available" phosphorus (8). The latter soils could be expected to give similar results to the high P soils in this series of experiments. A similar build up of phosphorus status on well established potato growing areas in the U.S.A. has been reported by Welch and Nelson from N. Carolina where 80 per cent of the soil samples tested were high or very high in available P and the estimated return for an additional 40 lb. P_2O_5 per acre was zero. Peech (9), Hawkins (10), Bryan (11) and Nelson and Hawkins (12) also present similar evidence and Bushnell (13) in Ohio, Connecticut and Maine on potato soils found the P fixing capacity of the soils had been saturated and

suggested that P applications could be reduced temporarily without reduction in yield.

Perhaps more important than the final yield figures where the crop had been allowed to mature is the effect of delay in tuber production (Fig. I) noticeable at all centres except No. 1 under moderate or high phosphate treatments. It is common practice in the potato growing areas, particularly in a wet season to cut down the life of the crop by spraying with herbicides to prevent blight from entering the tubers. In the current season (1958) this spraying occurred at or before the 16 week stage of growth. This must have resulted at some centres in even more serious loss of crop under moderate or heavy rates of superphosphate application, than if the potatoes had been allowed to mature.

The most important effect reported seems to be the relationship shown in Fig. IV between the uptake of phosphorus and the final yield of dry matter of tubers. Irrespective of soil and season, crop grown on the low P soils showed a positive correlation between these two factors and those grown on high P soils a negative one. Figures quoted by Prince (14) working on soils previously fertilised with different levels of superphosphate annually for 36 years seem to show a similar negative correlation for the high phosphate treated soils. The writers consider that there is good evidence of a phosphate toxicity in the plants giving rise to reduction in the production of tuber dry matter on high P soils under normal superphosphate fertilising practice in the area. The average rate of application of superphosphate for potatoes in two of the main potato growing counties of Scotland is 1.83 and 1.14 cwt. P_2O_5 per acre in addition to about 10-15 tons per acre of F.Y.M. (15) (16). This fertiliser is usually placed in the drill just below the seed, as was done in the experiment, giving rise to considerable local concentration of phosphorus as well as nitrogen and potassium. Under these circumstances on high P soils with low fixing powers it appears reasonable to assume the presence around

the plant roots of fairly high concentrations of P in solution.

There is a considerable amount of evidence that phosphorus even in solutions of fairly low concentration may be toxic to plant life. Williams (17) working with Phalaris tuberosa and oats under different phosphate treatments observed an initial depression in growth (a similar effect has been repeatedly observed by one of the writers, K.S., with oats and potatoes) and stated that "it seems that the synthesis of nucleo-protein is greatly retarded by high phosphorus supply. This in accord with a check in meristematic activity and in the dry weight increase". Williams' results show that this is not speculation. This observation fully agrees with the results reported here.

A possible explanation of the reductions in yield would be early season root damage associated with the high concentration of salts around the parent tuber. The writers consider this to be the exception rather than the rule. "Scorching" of roots was observed only at centres 2 and 3 under the highest rate of application, which is outside the normal range of fertiliser treatment whereas delay and reduction in tuber yield occurred much more frequently. Also plants might be expected to recover from an early season check as the concentration of salts in solution declined whereas the effects noted here usually lasted throughout the season.

Rossiter (18) working with subterranean clover and oats described symptoms of P toxicity. He reported toxic effects by as small a dressing as 2 cwt. superphosphate per acre on a very sandy soil but found that on less sandy soils up to 50 cwt. per acre of superphosphate was required to produce symptoms. Rossiter also stated that reductions in yield could occur without symptoms being observed. In this series of experiments the lowest yields of dry matter of tubers were produced on high phosphate sandy soils (see Table I) at centres 2 and 3, under high P fertiliser treatments in a wet season which according to Simpson (2)

increased the availability of fertiliser phosphorus.

According to Woodman and Johnson (19) the amount of phosphorus in a nutrient solution required to produce toxic effects is quite low. Using swedes in gravel soil cultures they found curves similar to those presented in Fig. I with an optimum yield at 8 p.p.m. of P in the nutrient solution falling off rapidly to 22 p.p.m., and then remaining approximately constant up to 90 p.p.m. Richards (20) found similar effects with barley where high levels of phosphorus were used in sand cultures, and reported further that the reduction of phosphorus uptake by adding rubidium to the nutrient solution improved growth and the general condition of the plants.

There is some evidence that, in small seed plants, phosphorus is quite toxic to seedlings. Moore (21) found that the PO_4 ion used at ordinary nutrient solution concentrations was injurious to peanut seedlings. Hamner (22) also considers that seedlings are more susceptible to P poisoning than older plants and postulates three possible reasons, "greater absorption of phosphorus at the seedling stage, lower tolerance for P or the amount of P stored in the seeds". In this series of experiments with potatoes early season toxic effects were not observed. It is suggested that the potato, which has no true seedling stage, is less susceptible to high concentrations of P than small seed plants.

The well established effect of nitrogen-phosphorus interaction on plant growth appears to play a part in the control of P toxicity. Lemmerman and Behrens (23) obtained satisfactory growth of oats in pots where the $\text{P}_2\text{O}_5:\text{N}$ ratio was 1:1 but not where it was 3:1. Hamner with soybeans also found the 3:1 ratio to be critical, any higher ratio of $\text{P}_2\text{O}_5:\text{N}$ giving toxic effects. Rossiter (18) also found phosphate toxicity symptoms in oats only when the nitrogen supply was low on a very sandy soil but found no positive effect of phosphorus on yield even when 4 cwt. Na NO_3 per acre was used. In this series of experiments the rate of nitrogen

application remained constant while the phosphorus rate was varied and a criticism may be that the P toxicity effects would not have occurred if higher nitrogen treatments had accompanied the high phosphate applications. The soils in the experiments, however, had all ^{been} ~~be~~ subject to good farming practice with considerable applications of F.Y.M. Centre 3 in particular had been treated for many years on a market garden scale and was very high in organic matter. The foliage on all plots was a healthy dark green colour and nitrogen deficiency was never observed. Also the results of both an earlier series (24) and more recent factorial experiments as yet unpublished strongly support the use of no more than 5 cwt. of ammonium sulphate per acre, as used in these experiments, irrespective of the amounts of phosphorus and potassium used. Higher rates of application tend to stimulate shoot growth at the expense of tuber production. It is felt therefore that, despite the use of as much as 112 lb. nitrogen/per acre on the high P soils the ratio of P:N in the soil solution is high enough to produce toxic effects when superphosphate is placed in the potato drill at rates normally used in commercial practice. This practice must give rise to considerable local concentrations of fertiliser in the drill.

It is possible from the data in Fig. IV to suggest that an optimum total uptake of P_2O_5 (12 week stage) for potato tuber production is around 30-35 lb. per acre. Higher levels may cause reduction in crops.

It is considered that the results presented above give further strong evidence in support of the suggestion previously made (3) for a drastic reduction in the rate of application of superphosphate to potatoes in high P soils in S.E. Scotland.

CONCLUSIONS

1. The population and number of sprouts per hill were little affected by added superphosphate.

2. Shoot yield was stimulated by dressings of superphosphate up to 2.0 cwt. per acre on low P soils. Root and tuber dry matter yield were not stimulated to the same extent and were not increased further by applications above 0.66 cwt. P_2O_5 per acre. On high P soils, superphosphate had little effect on shoot yield but depressed root yield at high levels of application.
3. The number of tubers per plant was increased and the percentage of dry matter in all parts of the plant was decreased on low P soils by superphosphate additions.
4. Lower yields of tubers were produced in the wet season of 1954 than in the dry one of 1955. The ratio of ware/seed was very much higher in 1955.
5. "Luxury" uptake of phosphorus amounting to some 20 lb. per acre of P_2O_5 occurred even on the low P soils at high levels of superphosphate application.
6. A positive correlation was obtained between maximum P_2O_5 uptake in the whole plant and the final yield of tuber dry matter in low P soils. In high P soils negative correlation was found.
7. It is suggested that phosphorus toxicity occurs when the level of uptake exceeds 35-40 lb. P_2O_5 per acre at the 12 week stage of growth. This resulted in delayed tuber development and reduction in final yields. The optimum P_2O_5 uptake by the whole plant for tuber yield appears to be 30-35 lb. P_2O_5 per acre.
8. Many soils in the potato growing areas of S.E. Scotland contain high or very high reserves of available P and it is suggested that high rates of application of superphosphate applied as at present in the drill will produce little extra yield and may reduce it.

ACKNOWLEDGEMENTS

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TABLE II

Effect of rate of application of superphosphate on yield of
dry matter of shoot and root in lb. per acre

Centre	Part of plant	Time in weeks	Rate of application of superphosphate in cwt. P_2O_5 per acre						L.S.D. P=0.05
			0	0.33	0.66	1.00	2.00	4.00	
1	Shoot	8	204	299	373	375	443	455	53
		12	635	1035	1280	1826	1790	2002	258
		16	528	120	1286	2049	1677	1778	300
	Root	8	118	128	134	115	116	120	N.S.
		12	176	197	237	313	294	255	69
		16	125	152	283	292	252	267	62
2	Shoot	8	93	97	126	115	116	81	18
		12	750	745	712	715	1002	1109	179
		16	1281	1461	1351	1328	1329	916	193
	Root	8	39	49	57	54	39	38	9
		12	98	123	159	171	168	103	23
		16	176	181	174	198	190	108	36
3	Shoot	8	1178	1351	1816	1639	1588	1441	235
		12	2227	2819	2982	3163	3276	3280	449
		16	2908	2238	1777	2647	2675	1767	N.S.
	Root	8	333	312	328	327	341	252	54
		12	351	478	459	409	429	347	61
		16	431	456	363	345	333	247	63
	1955		0	0.25	0.50	1.00	2.00	-	
4	Shoot	8	281	537	703	869	905	-	111
		12	510	662	964	1119	1277	-	141
		16	319	614	667	627	937	-	101
	Root	8	95	111	132	144	125	-	17
		12	94	123	142	151	142	-	19
		16	115	125	131	137	137	-	16
5	Shoot	8	463	650	589	621	616	-	78
		12	1325	1613	1515	1517	1584	-	195
		16	1035	1019	1110	1124	1124	-	N.S.
	Root	8	107	134	135	136	134	-	16
		12	186	198	183	177	177	-	N.S.
		16	304	340	391	344	385	-	49

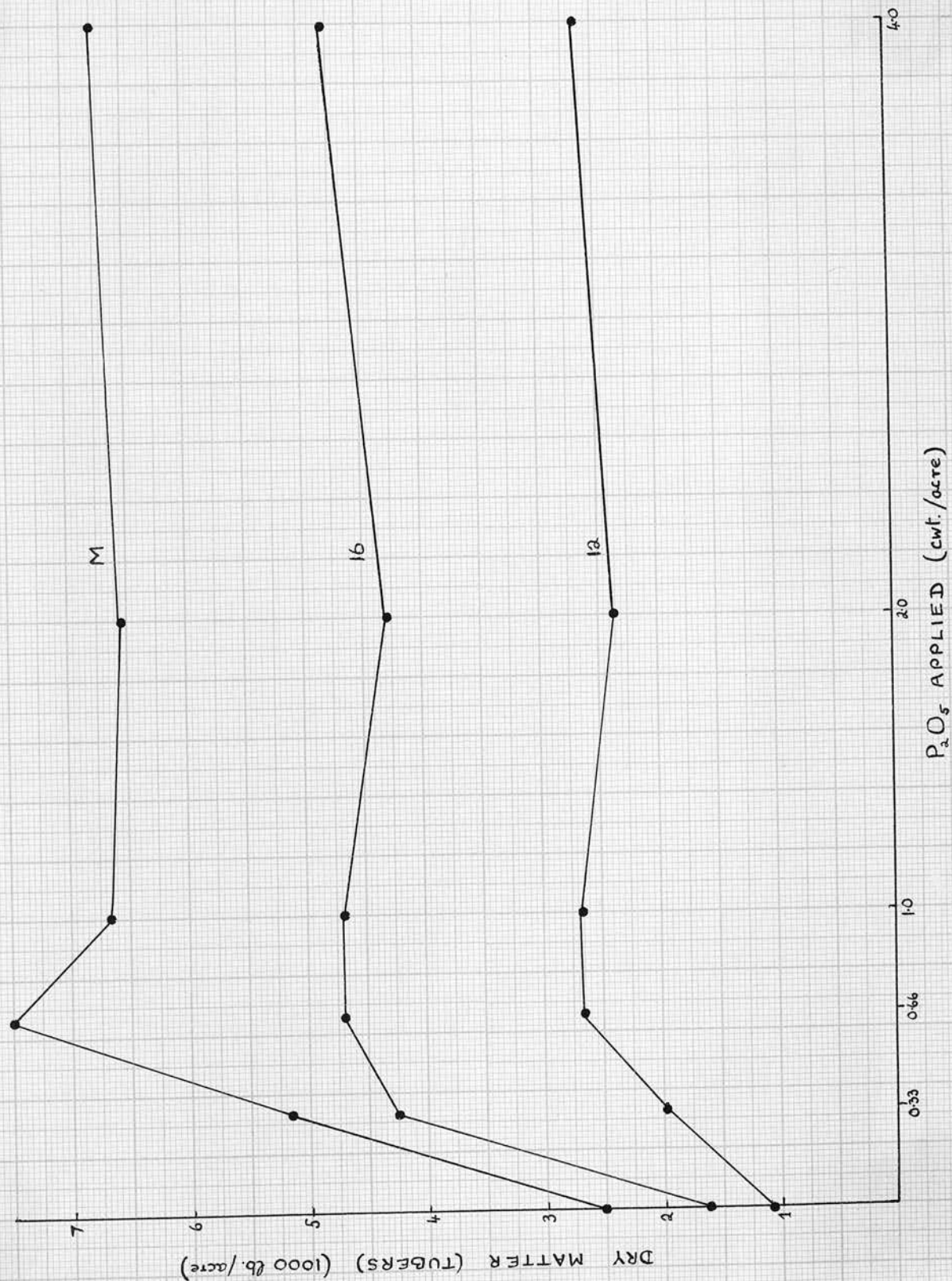
Fig. I. The effect of superphosphate on the yield of dry matter in the tubers at the 12 week, 16 week and maturity (M) stages at the five centres.

Centres I and IV : low available P

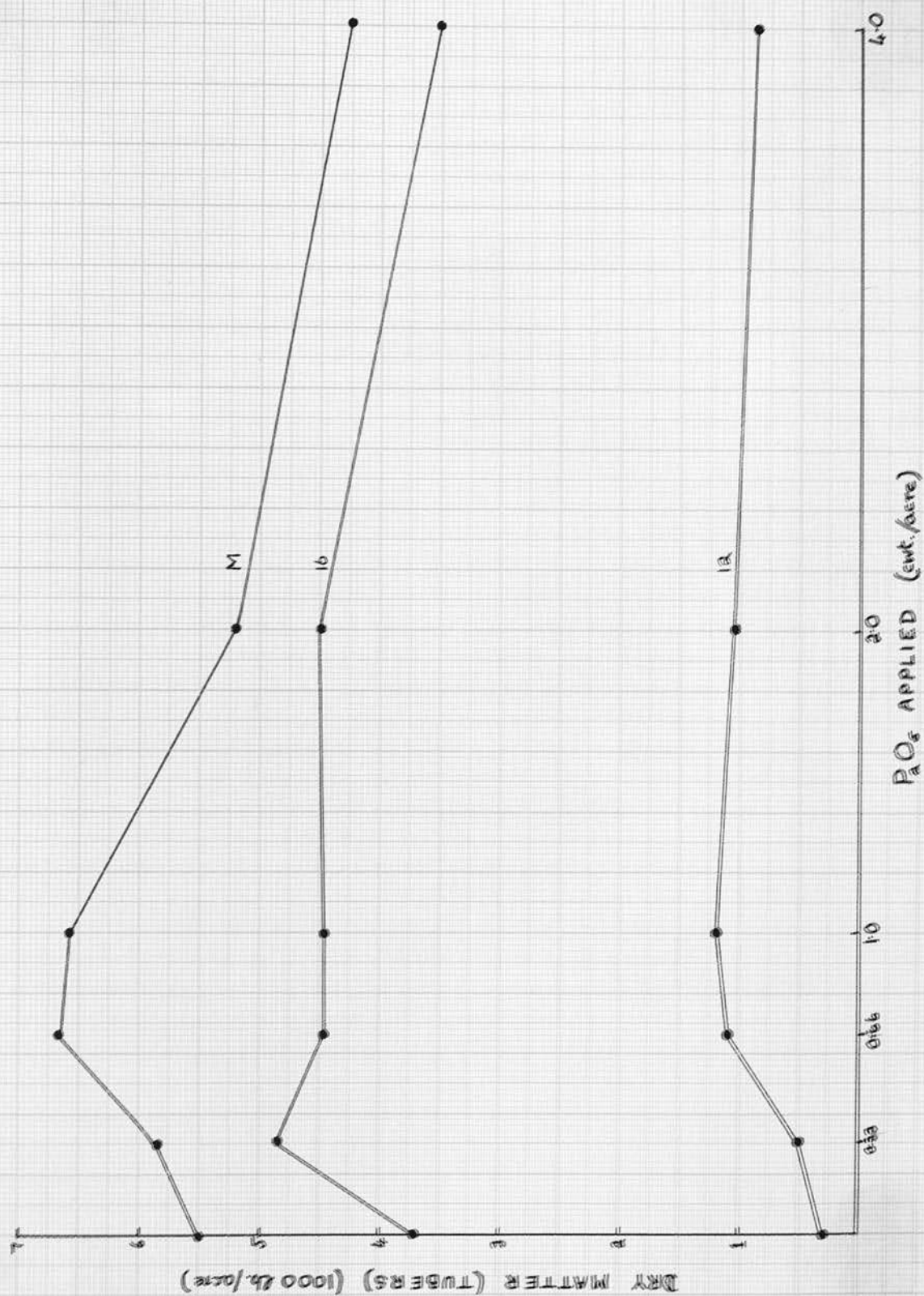
Centres II, III and V : low available P



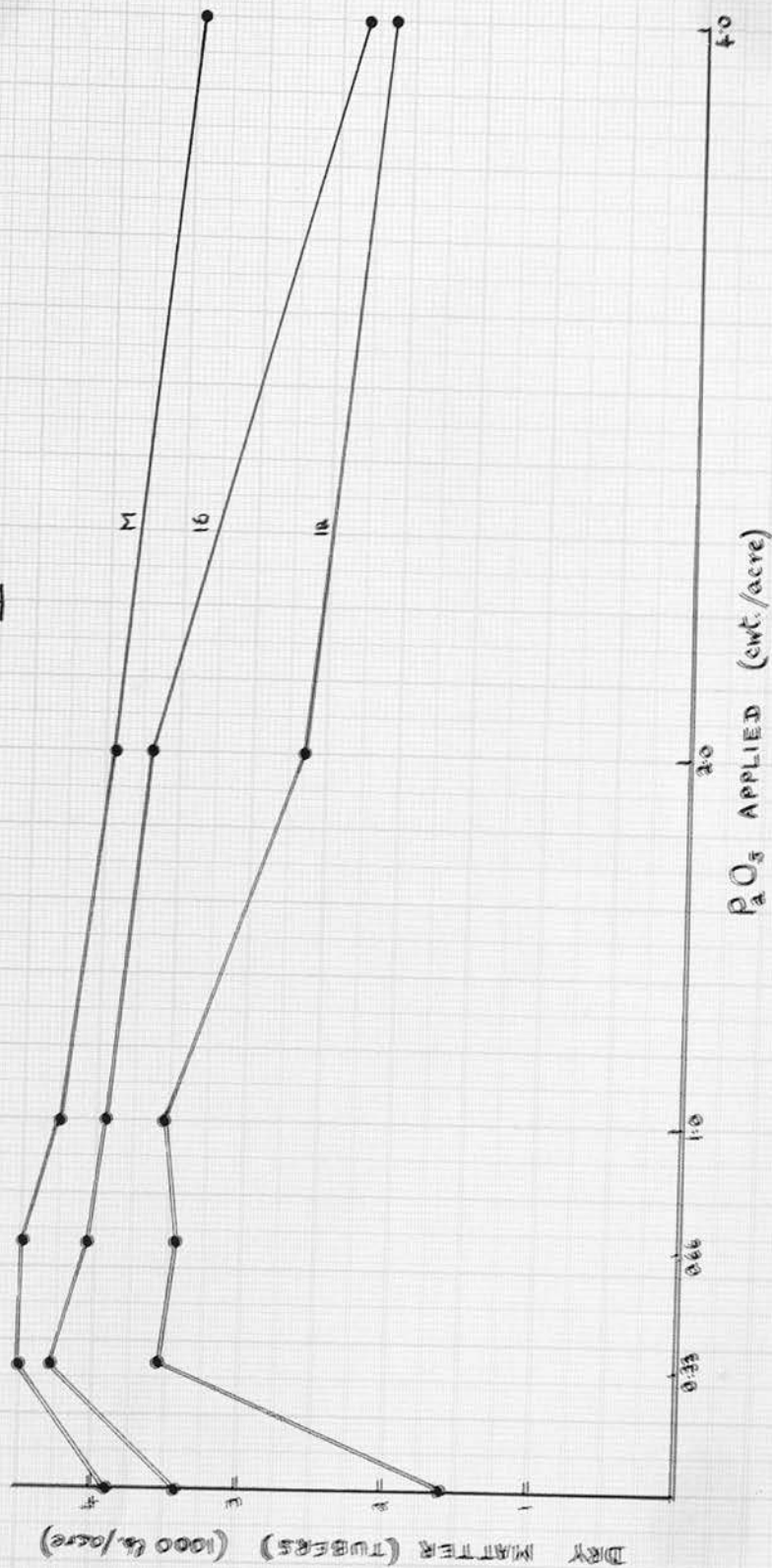
CENTRE I



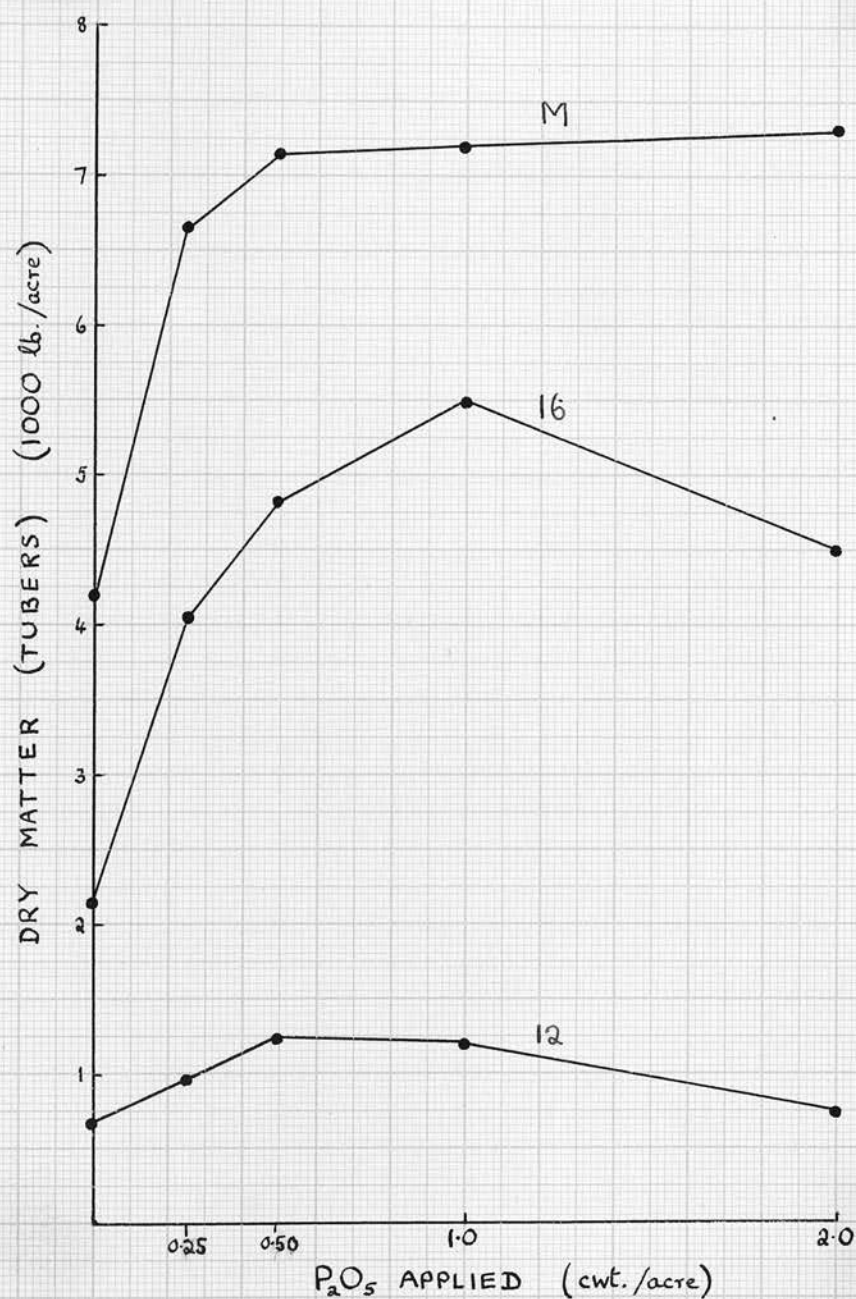
CENTRE II



CENTRE III



CENTRE IV



CENTRE V

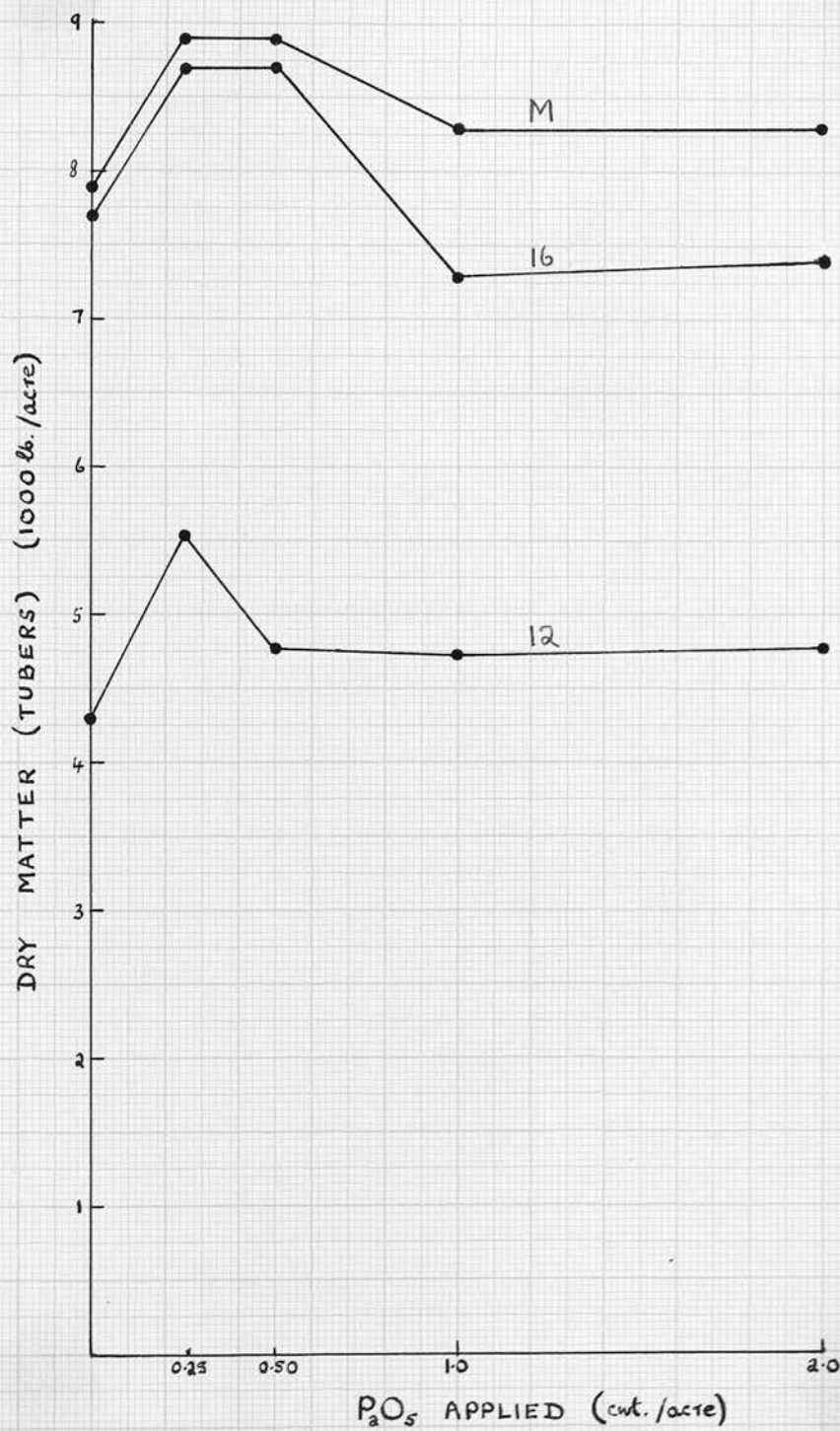
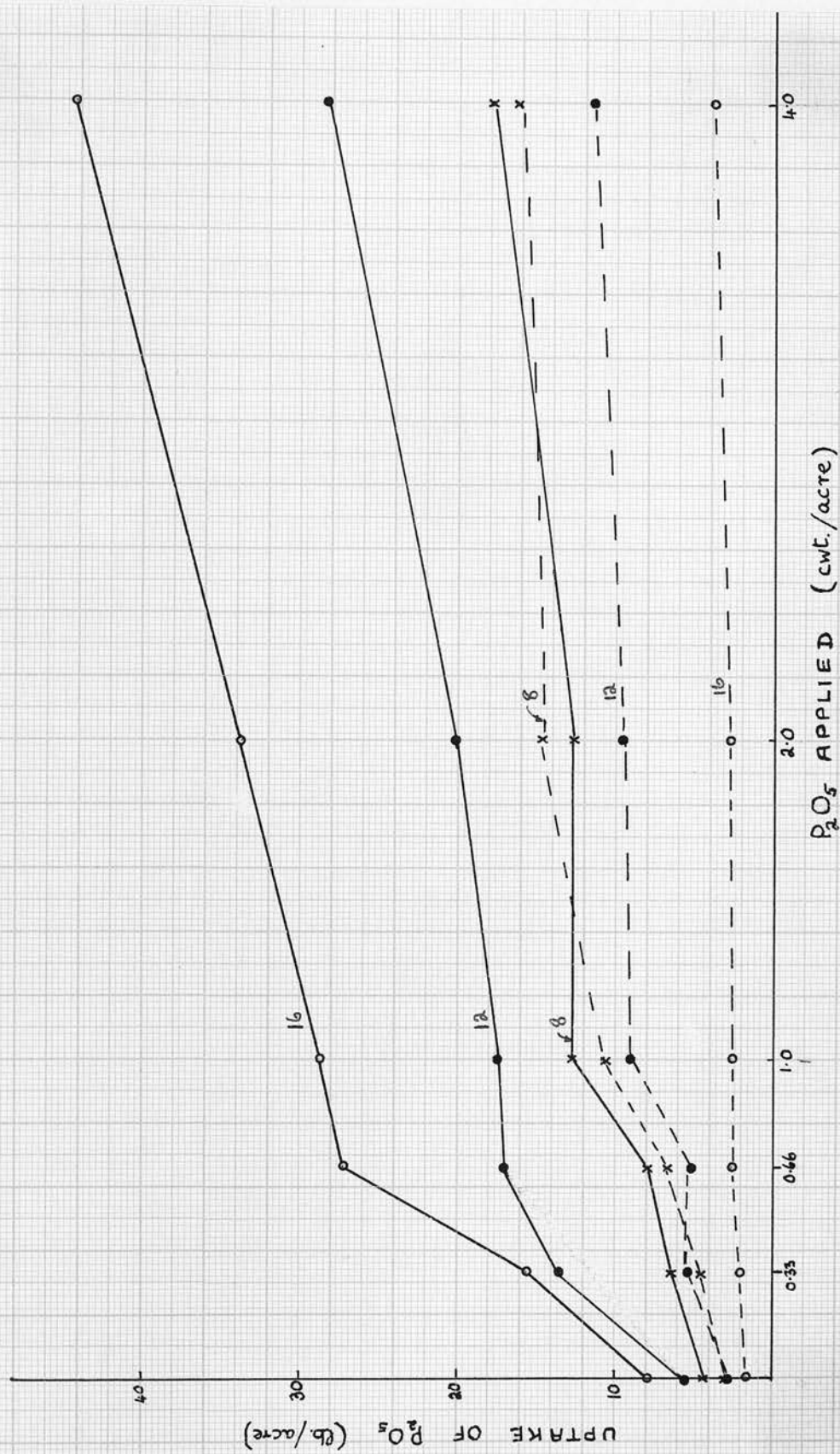


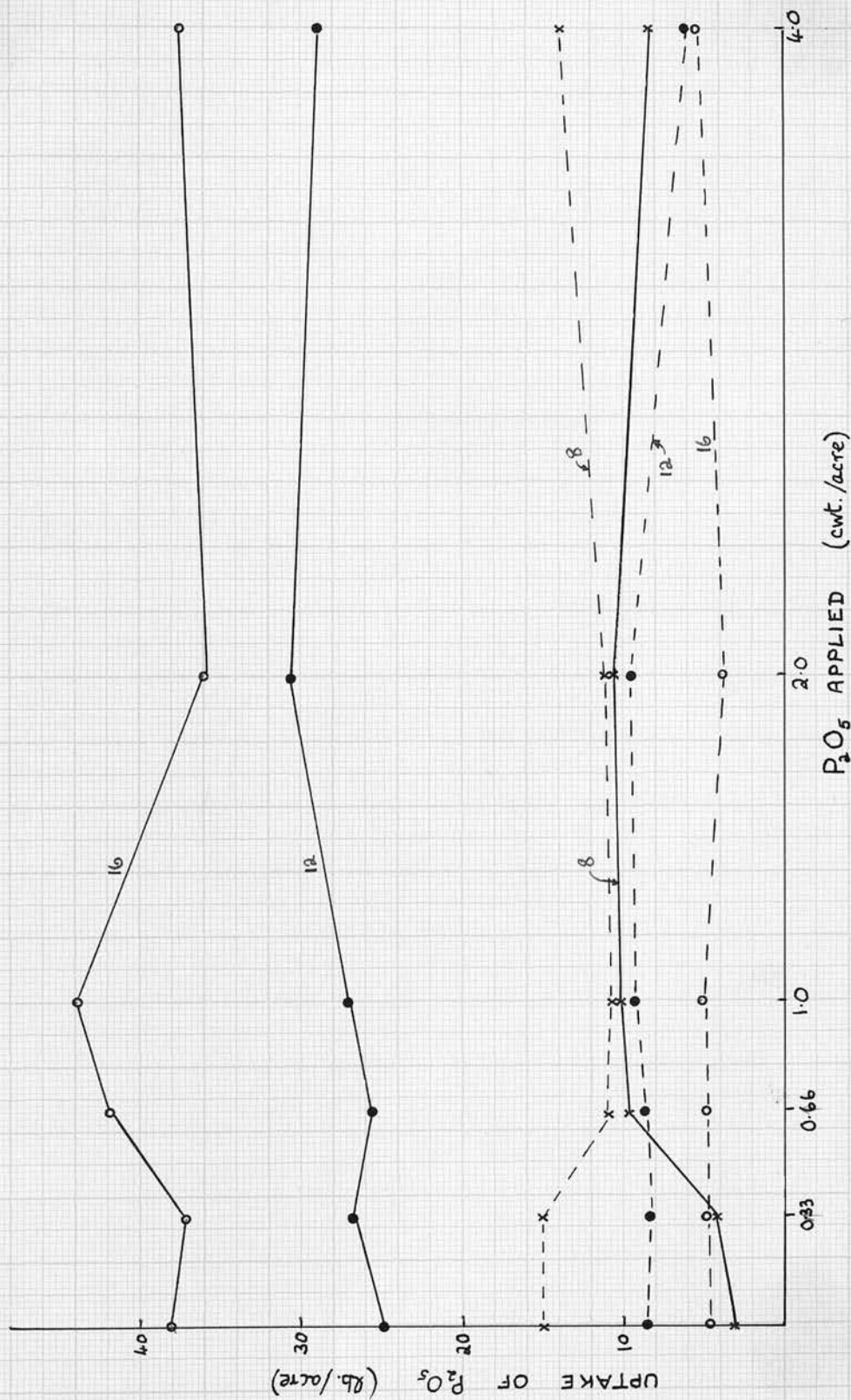
Fig. II. The uptake of P_2O_5 by shoot and (root + tuber), as affected by superphosphate treatments, at the 8, 12 and 16 week stages. Centres I to V as before.

----- shoot
——— root + tuber

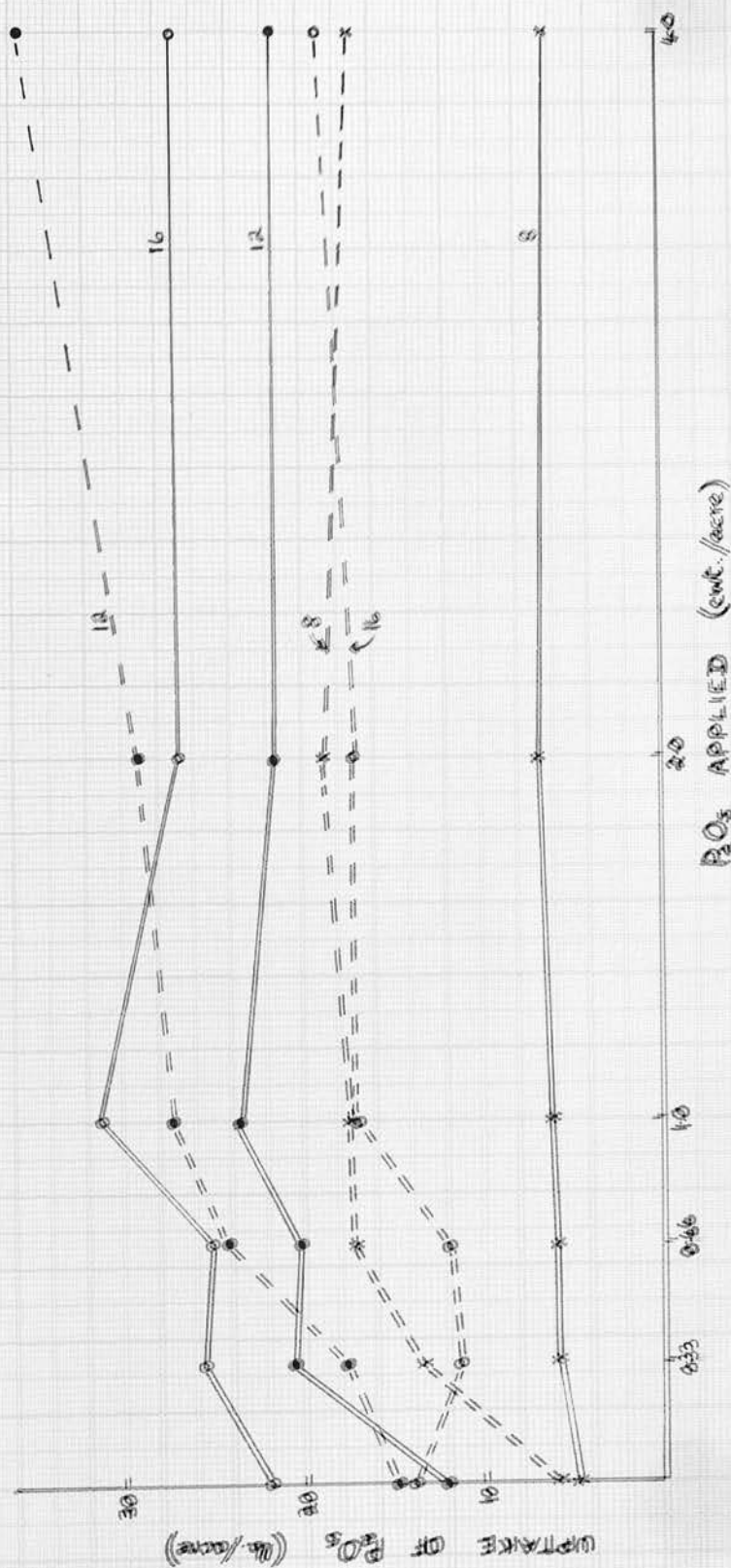
CENTRE I



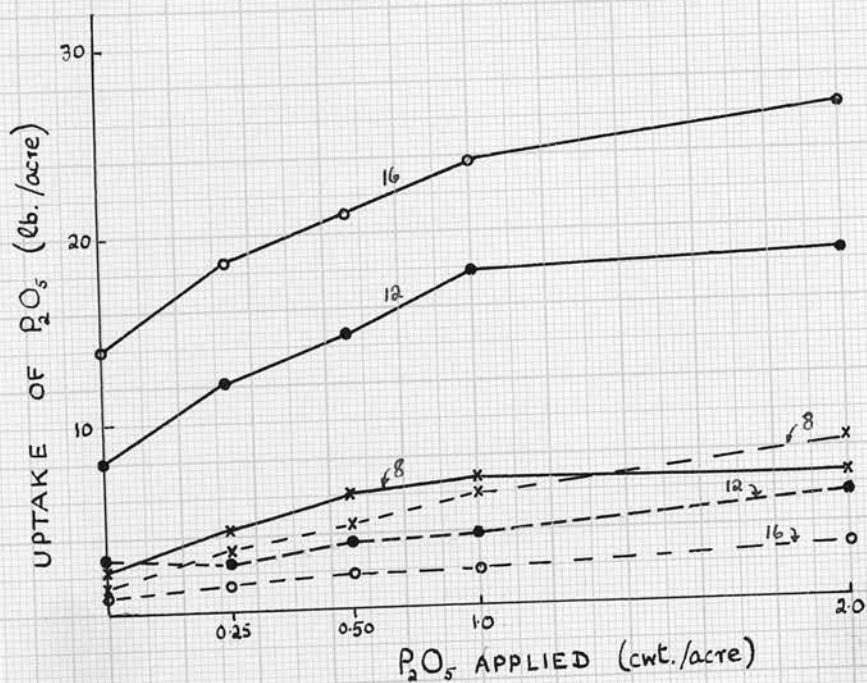
CENTRE II



CENTRE III



CENTRE IV



CENTRE V

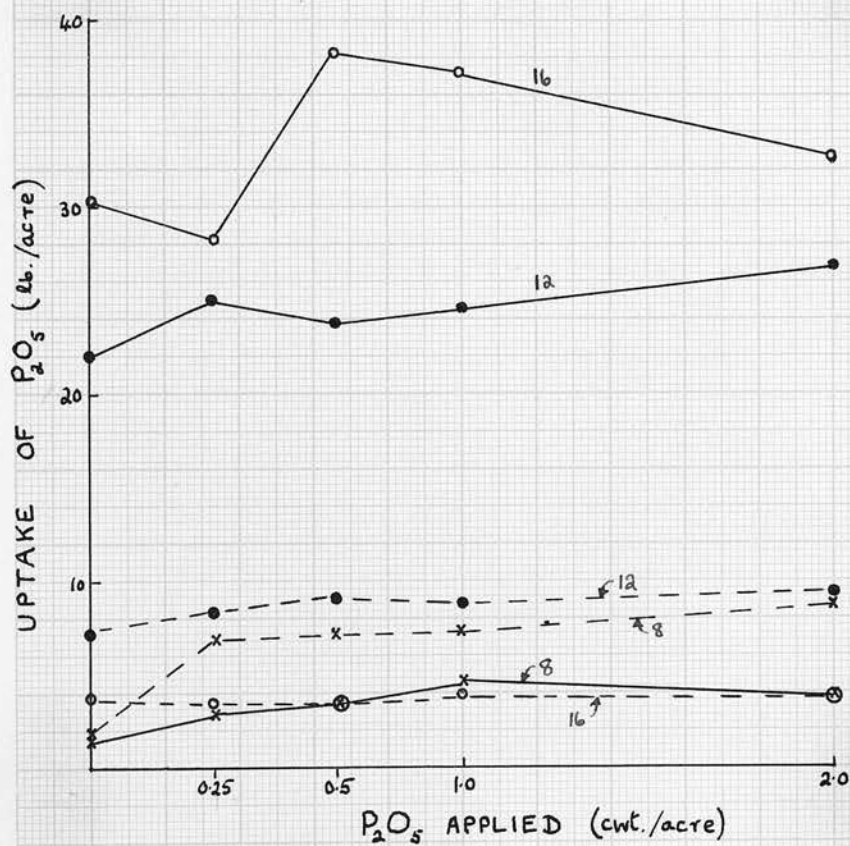
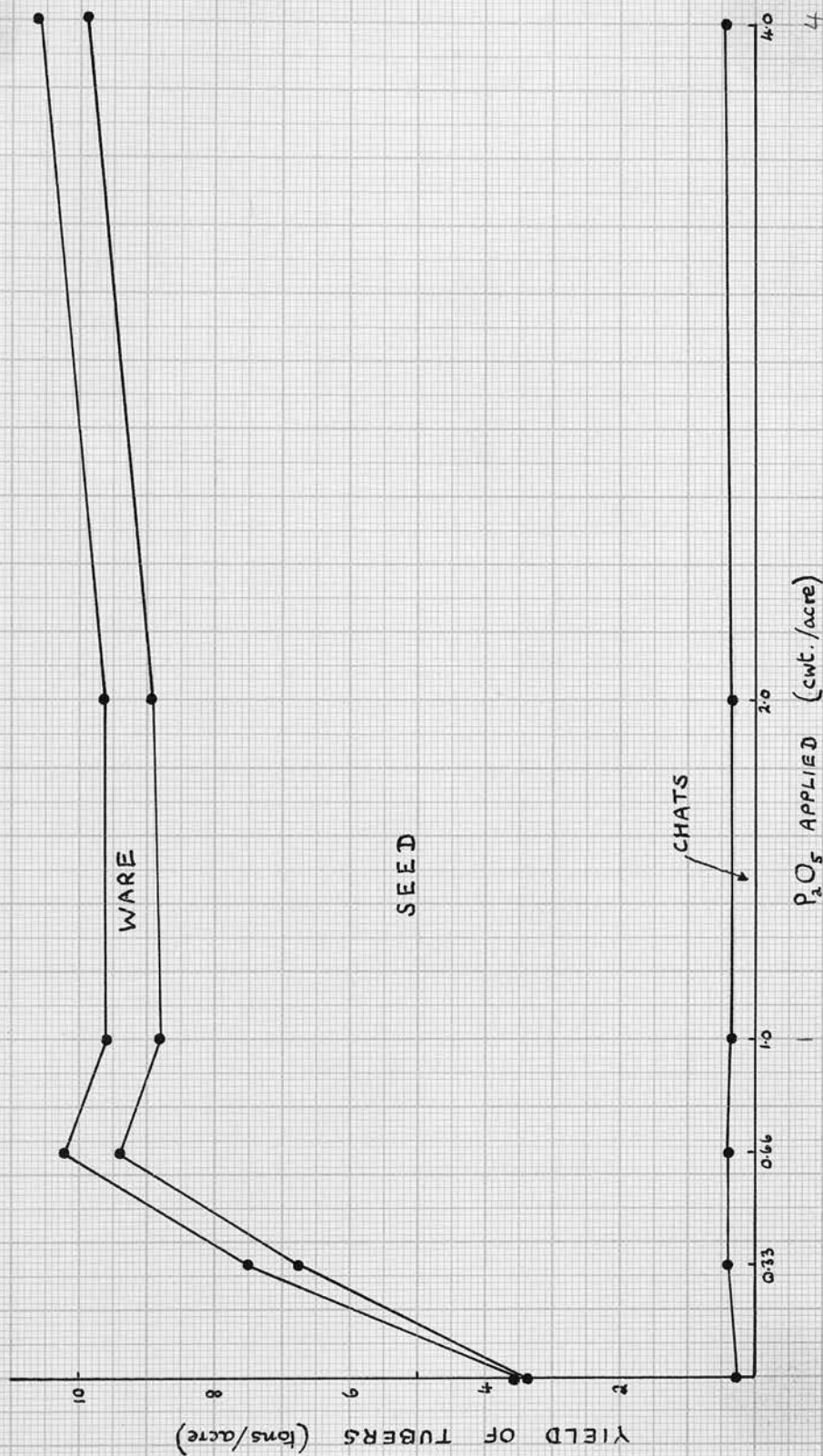


Fig. III. The effect of suberphosphate treatments on the yield of ware, seed and chaffs. Centres I to V as before,

CENTRE I



CENTRE II

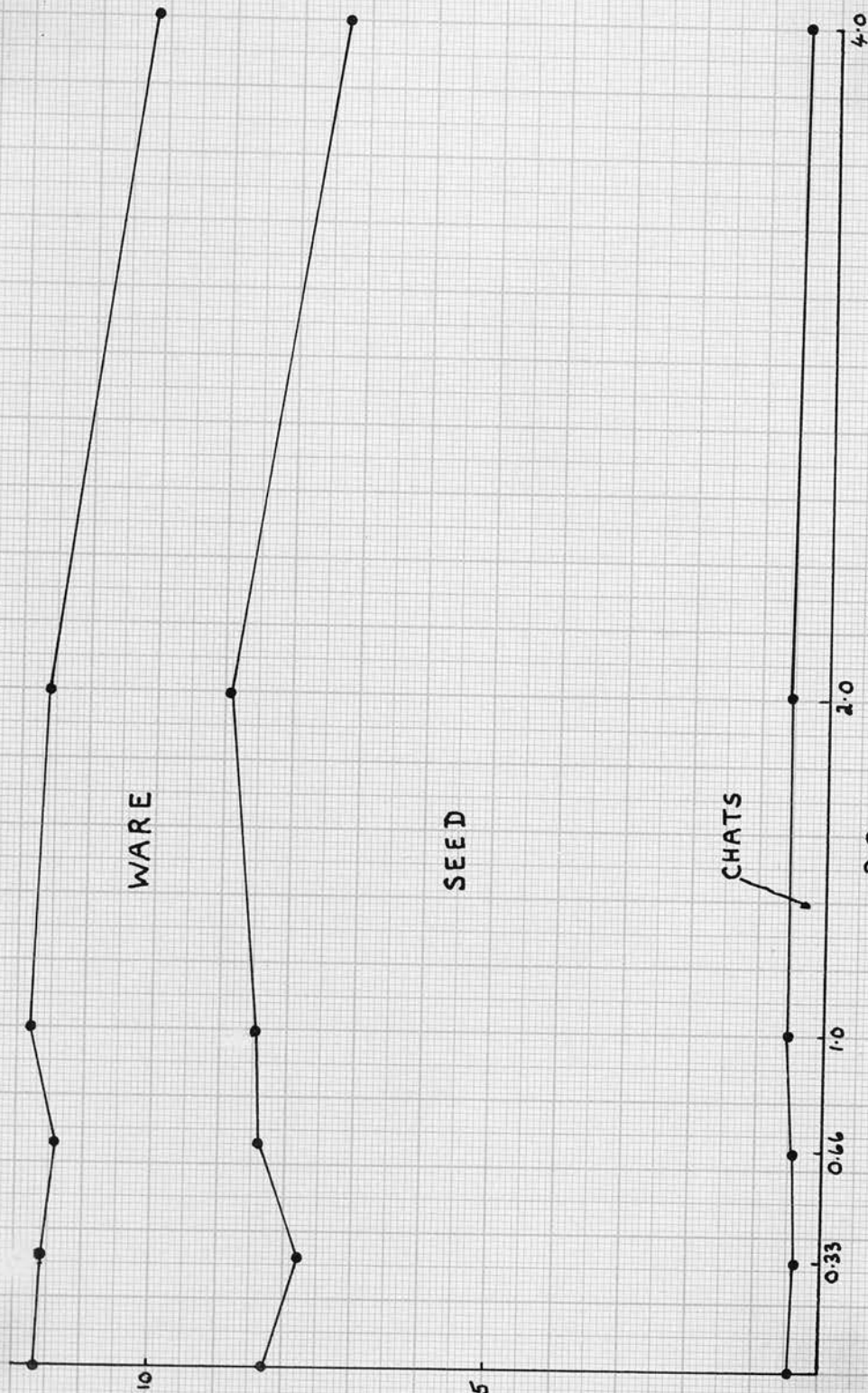
WARE

SEED

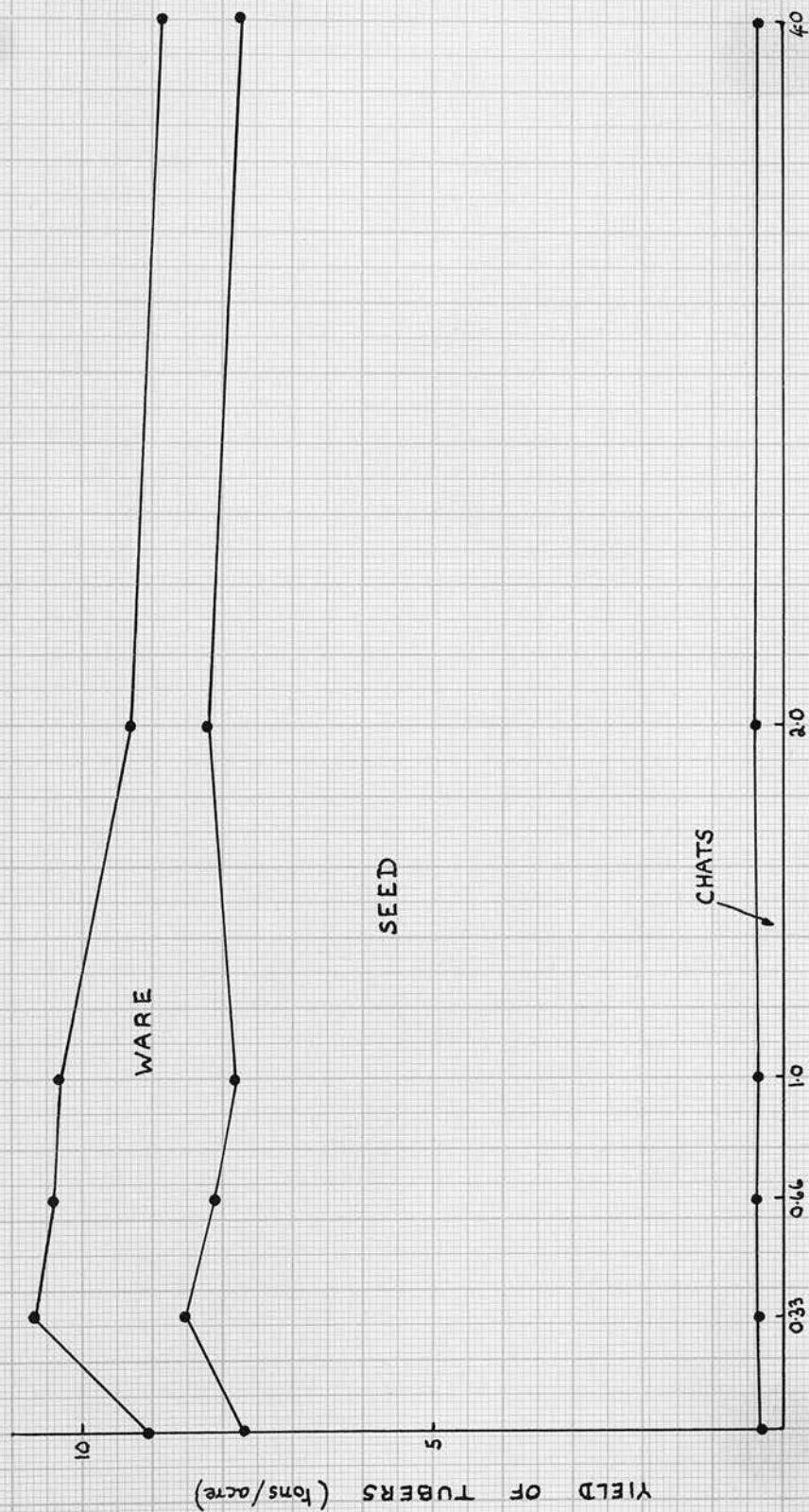
CHATS

P_2O_5 APPLIED (cwt./acre)

YIELD OF TUBERS (tons/acre)



CENTRE III



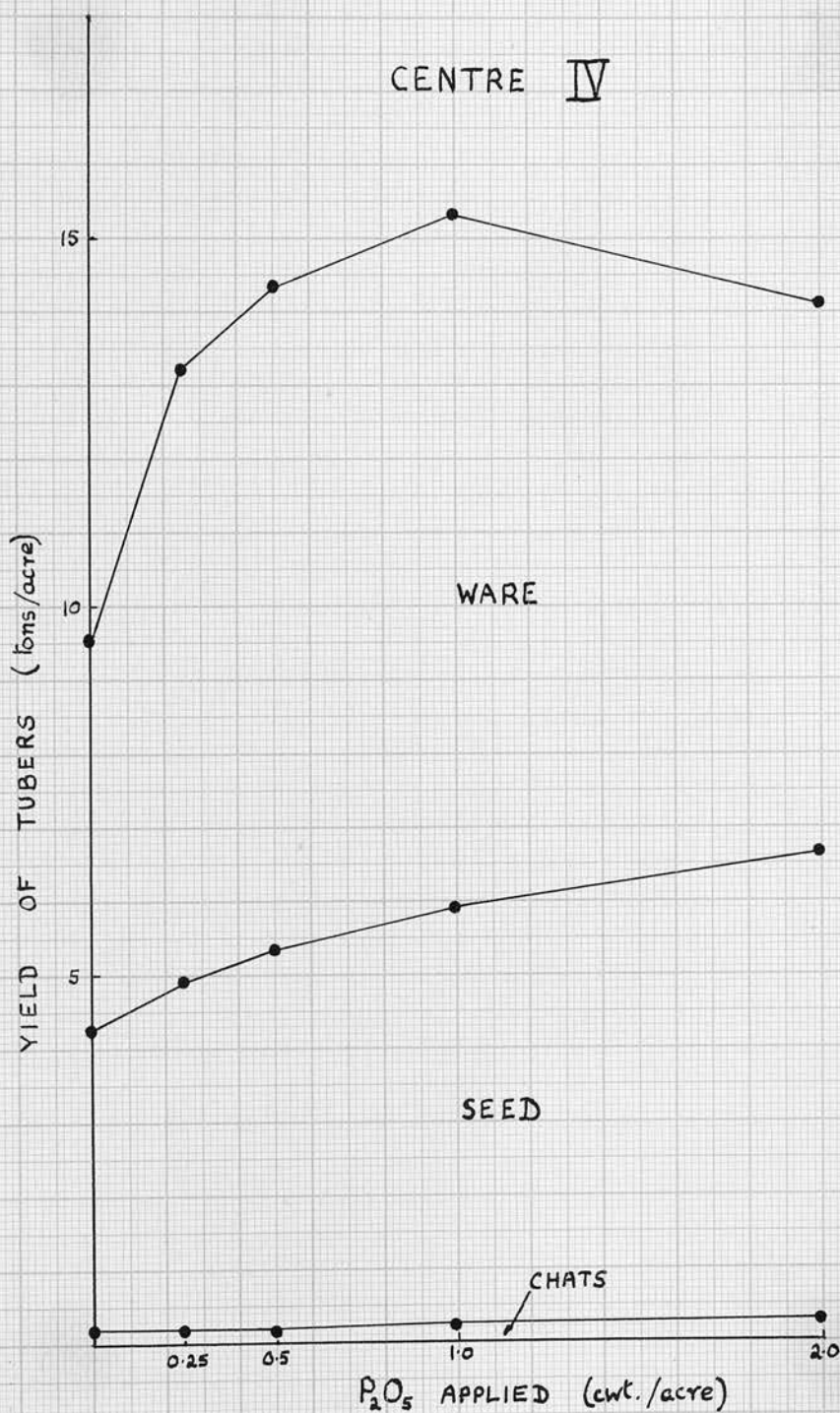
CHATS

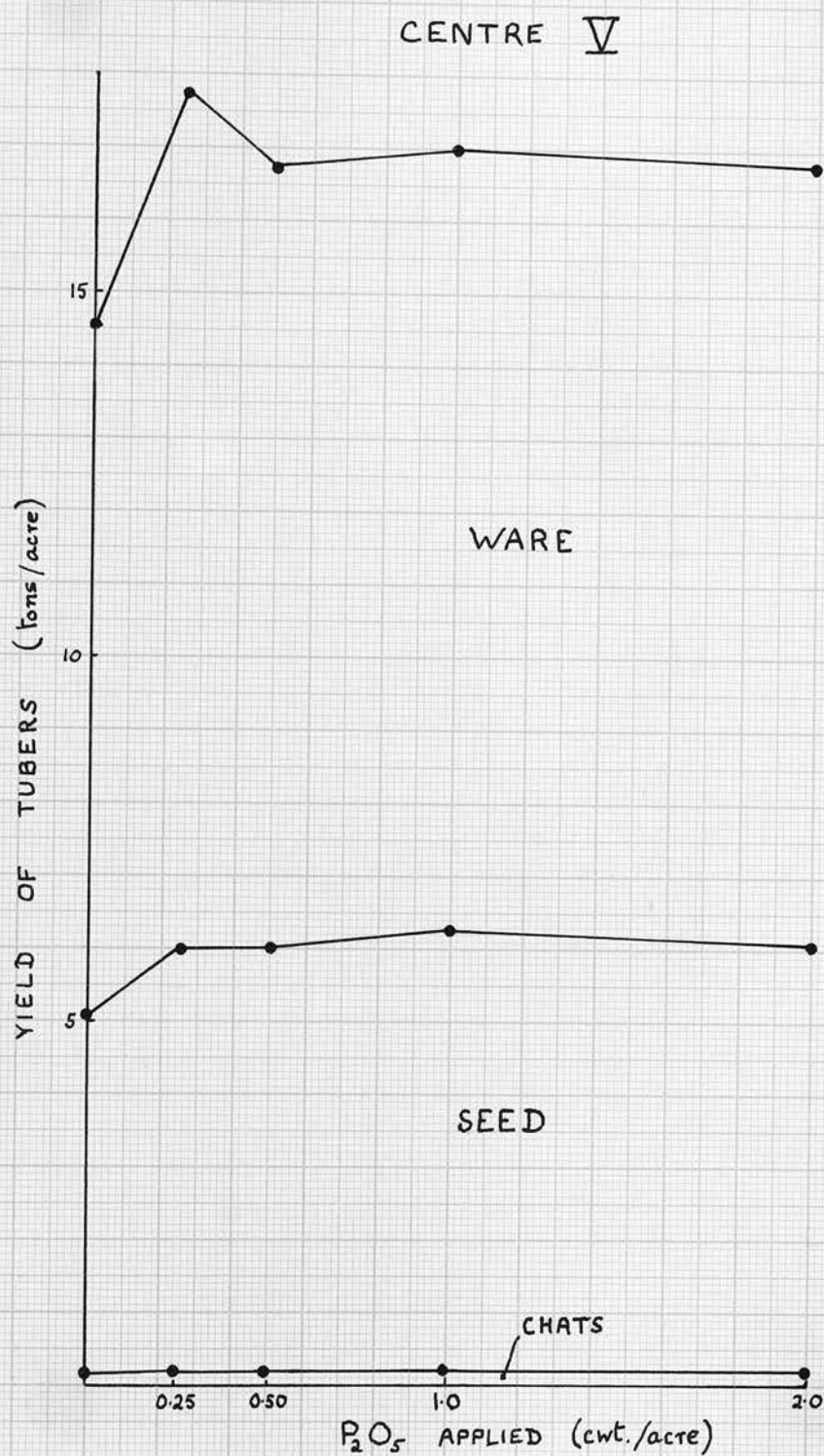
P_2O_5 APPLIED (cwt./acre)

YIELD OF TUBERS (tons/acre)

WARE

SEED





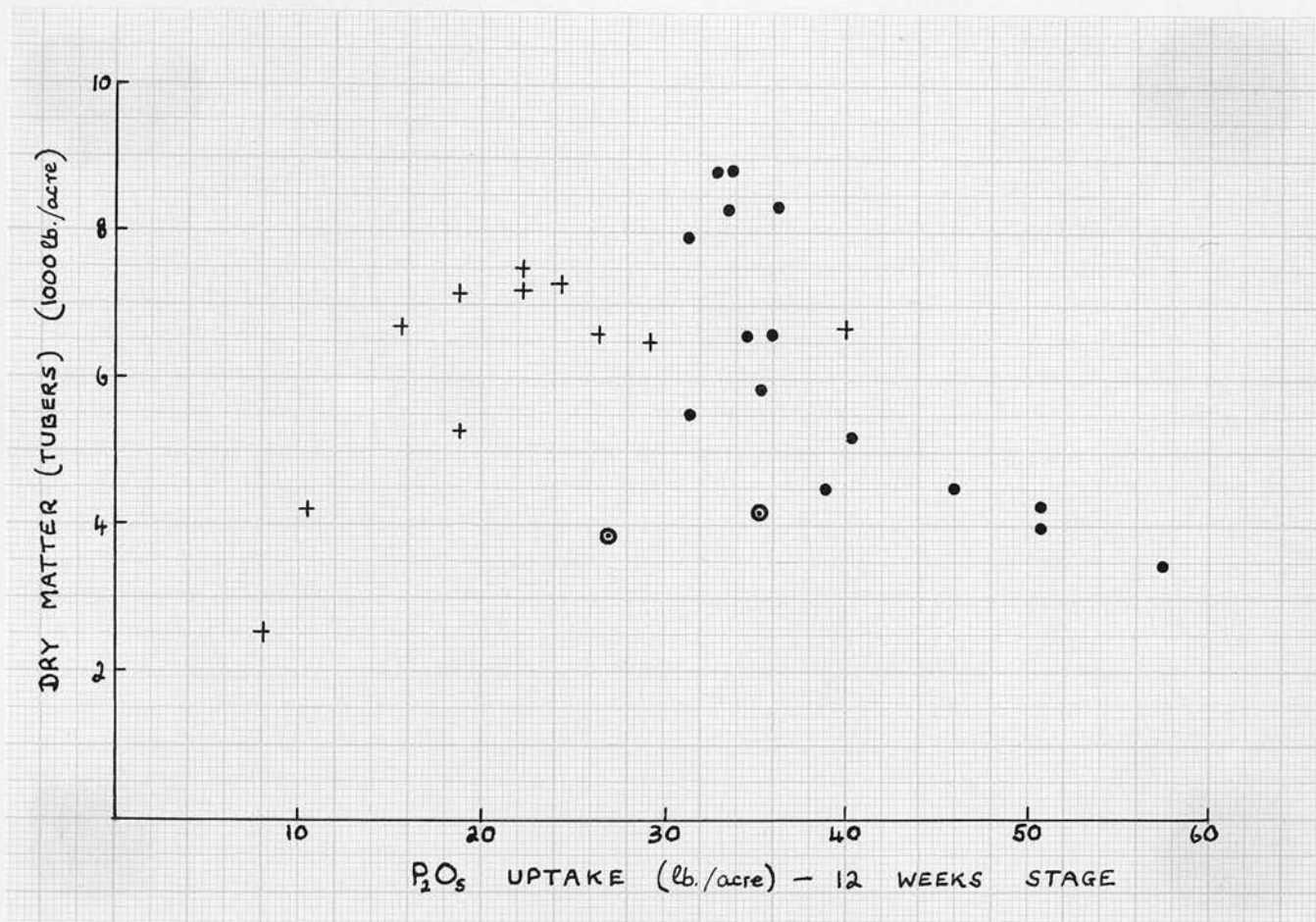


Fig. IV. The final yield of dry matter in the tubers plotted against the uptake of P_2O_5 .

crosses - low P soils

dots - high P soils

The two ringed dots are referred to in the text.